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IN VITRO AND *IN VIVO* EFFECTS OF AMINE BUFFERS

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INTRODUCTORY REMARKS

Gabriel G. Nahas
Department of Anesthesiology, College of Physicians and Surgeons, Columbia University, New York, N.Y.

The constancy of the internal environment of the body was defined over 100
ars ago by Claude Bernard[1] as one of the essential conditions for the mainte-
nce of life. Later, Cannon[2] coined the word "homeostasis" to define this same
ncept. One of the most important factors in homeostasis is the maintenance
a constant hydrogen-ion concentration in the body fluids.

Henderson[3] and Barcroft[4] defined the very narrow limits in which the *p*H of
od varied, mostly under the influence of a change in CO_2 pressure. Hender-
n[5] with Hasselbalch[6] devised a quantitative index for the measurement of the
id-base equilibrium in the blood based on the bicarbonate-carbonic acid
ffer system.

Considerable attention was directed towards the study of acid-base balance
blood and plasma, and this work was greatly facilitated by the techniques
vised by Van Slyke and Sendroy[7] and by the investigations of Singer and
stings.[8]

Many of these studies implied that plasma and extracellular fluid constituted
Claude Bernard[1] describes it: "the circulatory organic liquid which surrounds
d bathes all the tissue elements . . . and is diffused through the tissues and
ms the ensemble of the intracellular liquids." As an outgrowth of this con-
t, the acid-base status of the blood was equated with the acid-base status
the patient, and the terms of acidosis or alkalosis, though based on plasma
asurements, generally described conditions applied to the body as a whole.

Recently, Robin[9] pointed out the necessity of revising Claude Bernard's
ncept of the internal environment, since different body compartments are
arated by semipermeable membranes which limit the passage of ionized
rticles such as H^+ and HCO_3^-, and indirect *p*H measurements indicate that
*p*H in the cell is lower by 0.5 to 0.7 *p*H unit than the blood *p*H.[10] Therefore,
od *p*H may not be a good index of the *p*H prevailing in other body compart-
nts, and even in some cases it may vary in an opposite direction. Such
servations are of significant importance in view of the fact that the enzymes
ich trigger all the fundamental chemical reactions maintaining physiological
ivity, have different *p*H optima, and are quite sensitive to changes in ionic
ncentration.

t was when such concepts were being discussed, that an organic hydrogen-
acceptor, 2-amino-2-hydroxymethyl-1,3-propanediol, more commonly
led tris(hydroxymethyl)aminomethane or Tris buffer or THAM was used
McFarland[11] to control environmental *p*H in fish transport and, in our
oratory, by direct addition to the blood perfusing an isolated heart. The
essity of maintaining a normal or slightly alkaline *p*H of the blood perfusing
isolated heart to prevent cardiac failure was emphasized in the classic heart-
g preparation of Starling and Visscher.[12] In formulating "the law of the
rt," these investigators stressed the importance of maintaining "constant
mical conditions," for example, normal *p*H, if myocardial fiber length was

to be related to strength of contraction. In our experiments, THAM revers rapidly the failure of the isolated heart induced by equilibrating the bl perfusing the heart with elevated CO_2 gas mixtures, and the pH of the cir lating blood could be restored rapidly to normal. These results were cl cut, and they could not be duplicated with bicarbonate nor with lactate. decided then to use THAM in intact acidotic dogs[13] and observed that t compound, intravenously administered, exerted a marked buffering activ *in vivo*, relieving some of the physiological disturbances related to CO_2 retent in the body, to metabolic acidosis of severe muscular exercise, or to mass intravenous infusion of acids. Following these observations, experimen work as well as clinical trials with this compound have been carried out. addition to its buffering activity, it was shown that THAM exerts *in viv* marked osmotic and diuretic activity, depresses ventilation and, in large do has a hypoglycemic effect. The compound distributes widely throughout bo water and therefore penetrates into cells. The rate and extent of the distrib tion of THAM into cells is the subject of several reports. THAM was us clinically in some cases of acidosis where renal or cardiac lesions required restriction of the commonly used sodium buffers that equilibrate slowly w intracellular spaces. These pages include clinical reports from those who fi used this compound. All this work has justified publication of this monogra which describes results obtained in many areas by different investigators. publishing this monograph we have been fortunate enough to obtain contrib tions from seasoned investigators who analyze and discuss some of the fun mental problems related to the control of acid-base balance. These work have directed their attention especially toward the control of acidosis w amine buffers and especially with THAM.

In this publication physical chemists first discuss some of these fundamen problems that they study *in vitro*. We hear about the pH of these am buffers, the pK that determines their disassociation into ionized and no ionized moiety. We also learn more about the physicochemical characterist of these amines: such as heat-stability, hygroscopicity, solubility in water a oil. The use of alkali as emulsifying agents for the manufacture of soaps a detergents is widespread. That amine buffers, extensively used industrial should have similar properties is also of interest to the physiologist and pharmacologist. Today the metabolism of fat as it occurs *in vivo* will requ better understanding if a successful therapy of arteriosclerosis is to be fou Moreover there is certainly a need for an internal detergent that can slow do or prevent the clogging of some of the important vessels that go to the he and the brain. In this respect we include studies performed with surface-act agents in the production of microemulsions.

Fenn[14] demonstrated about 30 years ago that the potassium content of mus was dependent upon intra- and extracellular pH relationships. Since then t original observation has been extended to other electrolytes and to compou such as barbiturates that partition in ionized and nonionized moieties. T effect of pH changes produced by amines on ionic exchanges across oil layers cell membranes is therefore of very significant importance. How rapidly these amines penetrate into cells? Do they modify membrane permeabil to certain ions, to glucose, or to drugs? Passage of drugs across lipid barri

ld be predictable from the disassociation constant and the lipid solubility
e undissociated drug moiety. How many pharmacologists, however, are
olling the *p*H of their preparations when studying the effect of a drug?
hermore, do these amines affect electrical activity of a nerve cell? Do they
the membrane potential?

zyme chemists and biochemists discuss the effect of amine and other
rs on the kinetics of the complex chemical reactions that involve enzymes
can be observed *in vitro*. In addition to their *p*H effect, do these amines
interact with enzymes and to what extent?

l these fundamental studies are designed to explain some of the properties
ines observed *in vivo*. If amines penetrate into cells, what enzyme system
ld they affect? What ionic shift should be expected? What interactions?
should they alter the distribution or elimination of drugs?

e last part of this monograph is devoted to experimental and clinical
vations made in the course of THAM administration. Thus far this
ound is the only amine that can be used in doses sufficient to have a buffer-
ffect on the organism (3 to 500 mg./kg.) without any marked toxicity.
low toxicity of THAM is one of its most striking characteristics, since most
es produce marked toxic effects *in vivo* when used in doses sufficient to
a buffering activity. The important problem of amine toxicity is the
ct of special reports.

is apparent that the availability of a hydrogen-ion acceptor that could
trate cells and modify intracellular *p*H in order to alter the activity of a
fic enzyme would be of considerable interest to the researcher. These
compounds may also be useful to the clinician who is trying to correlate
and more certain disease processes with enzyme activity. When more
ese correlations are established it will be apparent that the alterations of
cellular *p*H may be of significant benefit. In this respect, it is possible
the hypothesis of Warburg,[15] relating malignant growth to anaerobic
bolism and lactic acid accumulation, could be tested by the use of such
ounds. We therefore hope that the organic chemist will be able to provide
th a series of hydrogen-ion acceptors that will have a *p*H range from 7.4
8 and will be able to penetrate into cells rapidly and act there as intra-
ar buffers. These compounds should be stable in body fluid and should
a small equivalent weight in the vicinity of 100 and a low toxicity. It
l be desirable also that these compounds be effective when given orally
ad of intravenously. The *p*H of the gastrointestinal tract varies from 1
), a *p*H range that will produce an ionization of any buffer well below its
ive range in body fluids. Furthermore, as shown by Hogben,[16] the in-
al mucosa preferentially allows the absorption of the un-ionized form of
or chemicals.This explains why THAM, when given orally, acts as a
rtic. In order for such compounds to be effective orally, it would be
sary that their hydrogen-ion accepting radical be inoperative during
ure to the acidic environment at the intestinal blood barrier, and then
red to activity after passage into the blood stream. The making of such
gen-ion acceptors would be difficult, but difficulty is for the research
ist more of a challenge than an obstacle.

The hope is that this monograph will stimulate a few investigators into t ing some of the hypotheses formulated in its pages

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rt I. Physicochemical Properties of Amine Buffers

AMINE BUFFERS FOR *p*H CONTROL

Roger G. Bates
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Introduction

ery few weak acids with dissociation constants between 10^{-7} to 10^{-9} are
wn. With the exception of a few phenols, only metaboric acid, barbituric
l, bicarbonate ion, and biphosphate ion have strengths lying in this range.
nsequently, acid-salt buffer systems suitable for the regulation of *p*H in the
ysiologically important range *p*H 7 to 9 are not numerous.

urthermore, most of these buffer solutions are incompatible in one way or
ther with physiological media. Phosphates and carbonates precipitate
cium salts. The usefulness of barbital (Veronal) buffers is limited not only
the low solubility of barbituric acid but also by a chemical inhibition of
tain enzyme systems.[1] The antiseptic properties of phenols usually prohibit
use of these substances for *p*H control in physiological media, while the
l-known tendency of borates to react with hydroxyl compounds is likewise
en a disturbing factor.

means of avoiding these difficulties has been sought through the use of
rogen bases and their salts in the hope that these buffers would be capable
egulating *p*H in the range 7 to 9 and, at the same time, would be compatible
h most physiological fluids. Buffer solutions consisting of ammonium salts
h added alkali are not highly stable. Kirby and Neuberger[2] and Mertz
Owen[3] accordingly suggested the use of imidazole (glyoxaline) and its
stituents as buffers. Although this substance is compatible with calcium,
relatively high cost has in the past greatly limited its use.

riethanolamine is a base of about the desired strength,[4,5] and triethanol-
ine-HCl buffers are accordingly useful for *p*H control in the range 7 to 9.[6]
s amine is a liquid of very high boiling point, however, and a pure product
ften difficult to obtain.[7]

n 1946, Gomori[8] proposed 3 new buffer systems suitable for *p*H control in
range 6.5 to 9.7. One of these is a liquid, 2,4,6-trimethylpyridine (col-
ne), and the other two are solids at room temperature; they are 2-amino-2-
droxymethyl)-1,3-propanediol[tris(hydroxymethyl)aminomethane] and the
ely related substance 2-amino-2-methyl-1,3-propanediol. Collidine and
s were found to be satisfactory in studies of the oxygen uptake of rat kidney
es; furthermore, they showed no inhibitory action on enzymes. The third
ne did not inhibit alkaline phosphatase at *p*H 9.1.

hese three compounds possess most of the characteristics of ideal physio-
cal buffer substances. They are readily soluble in water, do not precipitate
ium salts, and are low in price. Their solutions have been found to be
le at room temperature for periods of more than three months when pro-
ed from contamination with atmospheric carbon dioxide.

ith the discovery by Nahas and his associates[9-11] of its uses in the *in vivo* correction of respiratory acidosis, tris(hydroxymethyl)aminomethane achieved an importance that could never have been envisioned when this co pound was first proposed for *p*H control. Likewise, the use of the carbon and lactate salts of Tris in the treatment of metabolic acidosis has been s gested recently.[12] Tris has been utilized successfully in studies of blood coa lation[13] and in calorimetric investigations of the hydrolysis of adenosine triph phate[14] and of poly-L-lysine.[15] Furthermore, this versatile base can be obtai in a state of high purity and in this form is suitable as an acidimetric sta ard.[16,17] The complex formed by silver ion and Tris serves as a titrant for determination of sulfhydryl groups in proteins.[18] In view of these manif uses of Tris, the basic strength of this amine and related thermodynamic c stants have been measured over a wide temperature range,[19-22] and the values of Tris-hydrochloric acid mixtures have been determined.[23]

It is the purpose of this paper to identify the most important elements t determine the effectiveness of an amine buffer for *p*H control. In additi the properties of the most common of the useful amine buffers will be su marized as an aid to the selection of the most suitable substance for each par ular application. The discussion will be limited chiefly to monoacid ami with brief mention of suitable diacid amines and amino acids. Buffer syste will be considered from the following points of view: *p*H range; buffer val dilution value; change of *p*H with temperature; salt effects; and preparat and stability.

Range of pH

Water is an amphiprotic solvent, possessing both acidic and basic propert Consequently substances that are stronger bases than water are able to extr protons from water molecules (which are then behaving as acids); whereu the conjugate acid of the base, namely BH^+, is formed, together with hydro ion:

$$B + H_2O = BH^+ + OH^-.$$

In entirely analogous fashion, acids such as BH^+ are able to react to some deg with water molecules, which then function as a base:

$$BH^+ + H_2O = B + H_3O^+.$$

The extent to which these two reactions proceed is, of course, depend upon the relative strengths of the two bases concerned (B and OH^-) or of two acids (BH^+ and H_3O^+). The first reaction is referred to as the basic sociation of the base B. The equilibrium constant, termed K_b, is the ba dissociation constant of the base B. The second reaction is termed the aci dissociation of the acid BH^+. The equilibrium constant, termed K_{bh}, is acidic dissociation constant of the species BH^+. If B is an amine, the c jugate acid BH^+ is, of course, a substituted ammonium ion. It is easily s that K_b and K_{bh} are interrelated by means of the ion product constant (au protolysis constant) for water, K_w:

$$K_w = K_b K_{bh}.$$

The acid-base properties of amine buffers are therefore given quantitative ression by means of the mass law. For this purpose, it matters not whether equilibria are expressed in the form of Equation **1** or Equation **2**. In view the emphasis upon hydrogen ion concentrations, however, it is customary to the latter formulation and to characterize amines in terms of the thermo- namic dissociation constants, K_{bh}, of their conjugate acids.

f pH is given the formal definition

$$pH \equiv -\log a_{H} = -\log a_{H_3O^+}, \qquad (4)$$

mass-law expression for **2** assumes the following logarithmic form,

$$pH = pK_{bh} - \log \frac{m_{BH^+}}{m_B} - \log \frac{f_{BH^+}}{f_B}, \qquad (5)$$

ere pK_{bh} is written for $-\log K_{bh}$. The symbol m represents the molality the species indicated by the subscript, and f is the activity coefficient on the lal scale.

TABLE 1
VALUES OF THE DEBYE-HÜCKEL SLOPE (A) FROM 0° TO 60° C.

Temperature (°C.)	Slope A	Temperature (°C.)	Slope A
0	0.4918	30	0.5161
5	0.4952	35	0.5211
10	0.4989	40	0.5262
15	0.5028	45	0.5317
20	0.5070	50	0.5373
25	0.5115	55	0.5432
		60	0.5494

ata from Robinson and Stokes.[25]

n **5**, pK_{bh} characterizes the strength of the ammonium ion conjugate to the e; for a given buffer system it is a function only of temperature. The second m on the right, the buffer-ratio term, largely determines where the pH of buffer solution will lie with respect to pK_{bh}. It fixes, as well, the buffer acity.

The third term on the right, the activity-coefficient term, embodies the effect lifferences in the ionic strength or concentration of the buffer solution; it is strongly influenced by changes of temperature. For most purposes, suffi- tly accurate values of the activity coefficients can be computed by the owing modified form of the Debye-Hückel equation:[24]

$$-\log f_i = z_i^2\left[\frac{A\sqrt{I}}{1+\sqrt{I}} - 0.1I\right]. \qquad (6)$$

6, z_i is the charge of the species i and I is the ionic strength ($\frac{1}{2}\sum m_i z_i^2$). Debye-Hückel slope A is a function of the temperature and the dielectric stant of the solvent. Values of A from 0° to 60° C. are given in TABLE 1.[25] nasmuch as highly pure salts of amines are not generally available, amine fers are almost universally prepared from a weighed or measured quantity

of the amine or its solution, together with a measured quantity of a standa
solution of a strong acid. In general, 25 to 75 per cent of the amine is ne
tralized. Under these conditions, the buffer-ratio term lies between −0.5 a
+0.5, and the *p*H of the solution is accordingly fixed in large part by the mag

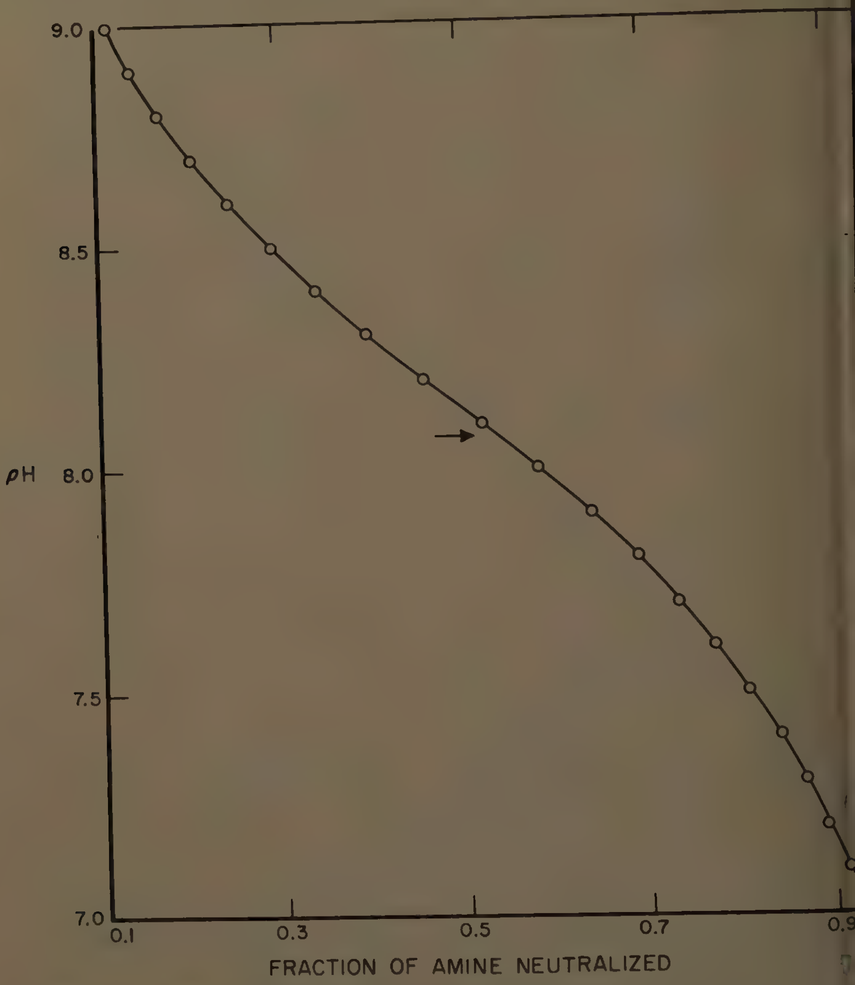


FIGURE 1. Range of *p*H of a typical amine buffer: *p*H of solutions composed of tris
droxymethyl)aminomethane (B) and its hydrochloride (BHCl) as a function of the frac
of amine neutralized. [B] + [BH$^+$] = 0.05 *M*, temperature = 25° C. The arrow indic
the point at which $pH = pK_{bh}$.

tude of pK_{bh}. The change of *p*H with the fraction of amine neutralize
shown in FIGURE 1 for a typical buffer system, tris(hydroxymethyl)am
methane and its hydrochloride.

The values of pK_{bh} and the heat of dissociation for amine bases at 25
are summarized in TABLE 2. The fourth column of TABLE 2 gives the

mputed with the aid of pK_{bh}, of a 0.05 M solution of the corresponding
monium salt. This is, of course, the *p*H at the equivalence point in the
ration of a 0.1 N solution of the amine with a 0.1 N solution of a strong
neral acid.

A good buffer resists a change of *p*H when it is accidentally contaminated
th acidic or basic materials and when dilution occurs. If the acidic contami-
nt is a weaker acid than BH^+, or if the basic contaminant is a weaker base

TABLE 2

CONSTANTS FOR THE DISSOCIATION OF CATION ACIDS (BH^+) AT 25° C., *p*H OF 0.05 M SOLUTIONS OF B·HCl

Acid	pK_{bh}	$\Delta H°$ (kcal. mole^{-1})	*p*H (0.05 M B·HCl)	Reference
hylenediammonium (first step)*	6.848	10.87	4.2	26
idazole (protonated cation)	6.95	—	4.3	2
,6-Collidine (protonated cation)	7.4	—	4.4	8
ethanolammonium	7.762	7.99	4.6	5
mino-2-(hydroxymethyl)-1,3-propanediol tris(hydroxymethyl)aminomethane] (protonated cation)	8.075	11.38	4.8	22
mino-2-methyl-1,3-propanediol (protonated cation)	8.790	11.88	5.1	‡
mino-2-ethyl-1,3-propanediol (protonated cation)	8.80	—	5.1	19
thanolammonium	8.883	10.14	5.2	27
minopyridinium	9.114	11.25	5.3	28
monium	9.245	12.48	5.4	29, 30
anolammonium	9.498	12.08	5.5	31
hedrinium	9.544	10.79	5.5	32
mino-2-methyl-1-propanol (protonated cation)	9.691	12.89	5.6	19‡
methylammonium	9.800	8.81	5.6	33
ylenediammonium (second step)	9.928	11.82	5.7	26
ropylammonium	10.568	13.67	6.0	25†
thylammonium	10.624	13.09	6.1	33
ylammonium	10.631	13.58	6.1	34, 25
utylammonium	10.640	13.88	6.1	25†
ethylammonium	10.715	10.56	6.1	25†
nethylammonium	10.774	11.86	6.1	33
amethylenediammonium (second step)	10.930	13.91	6.2	26
thylammonium	10.933	12.77	6.2	34, 25, 26
eridinium	11.123	12.76	6.3	35

* Dissociative process: $BH_2^{++} = BH^+ + H^+$.

† Provisional data of D. H. Everett, quoted.

‡ Private communication from D. H. Everett.

n B, the impurity is essentially inert. A strongly acidic contaminant,
vever, will convert an equivalent amount of B into BH^+, altering the buffer
io accordingly. The opposite effect is caused by strongly basic contami-
nts.

t is evident from a consideration of **5** that adventitious contamination will
duce the smallest effect when B and BH^+ are present in equal amounts.
e numerical calculation of the buffer capacity (to be considered in the next
tion) shows that the buffering effect in moderately dilute solutions is satis-
torily high when the amount of unreacted amine lies between 10 and 90 per

cent. It is therefore customary to regard the effective pH range of a gi buffer system as two units, extending from 1 pH unit less than pK_{bh} to 1 unit greater than pK_{bh}.

It is sometimes advantageous to utilize the contiguous or overlapping ran of two or more buffer systems, as in the so-called "universal" buffers. T phosphate salts of the ethanolamines have been shown[36] to be capable of p ducing, in suitable combination, an approximately uniform buffer capac throughout the pH range 5.5 to 12.8. This application is successful larg because ethanolamine ($pK_{bh} = 9.5$) bridges the gap between the second a third dissociations of phosphoric acid ($pK_2 = 7.2$ and $pK_3 = 12.3$).

Buffer Capacity

The two most useful criteria of the effectiveness of a buffer solution for control are the buffer value and the dilution value; both can be given numeri expression.

The buffer value defined by Van Slyke[37] denotes the ability of a solution resist a change of pH upon the addition of strong acid or strong alkali. It given the symbol β and is defined as follows:

$$\beta \equiv \frac{db}{dp\mathrm{H}}.$$

Evidently, β is the slope of the plot of b, the number of gram equivalents alkali (added to 1 l. of the buffer solution), as a function of the pH. An inc ment of strong acid is equivalent in effect to a negative increment of strong b and produces a decrease in pH; hence the ratio $db/dp\mathrm{H}$ is always positive.

The buffer value can be determined experimentally, and it can also be cal lated in a useful way from theoretical considerations. The following deri tion is essentially that of Van Slyke.[37]

Consider a buffer solution composed of amine hydrochloride, BHCl (m and the free amine, B (xm_1). The total concentration of amine and its c jugate acid in the solution is $m_1(1 + x)$ and will be denoted simply M. Wh b gram equivalents of strong alkali are added to a volume of the buffer solut containing 1 kg. of water (that is, approximately 1 l.), the solution must rem electrically neutral; hence:

$$b = m_1 - m_{\mathrm{BH}^+} - m_{\mathrm{H}} + m_{\mathrm{OH}}.$$

The molality of BH$^+$, which appears in **8**, can be expressed in terms m_1 and K_{bh} by means of the mass law:

$$\frac{m_{\mathrm{H}} m_{\mathrm{B}}}{m_{\mathrm{BH}^+}} = K_{bh} \frac{f_{\mathrm{BH}^+}}{f_{\mathrm{H}} f_{\mathrm{B}}} \approx K_{bh}.$$

The activity-coefficient term is, to a good approximation, unity in dilute s tions. From **8** and **9**, together with the approximate ion product constant water, $K_w \approx m_{\mathrm{H}} m_{\mathrm{OH}}$, one obtains

$$b = \frac{m_1(K_{bh} - x m_{\mathrm{H}})}{K_{bh} + m_{\mathrm{H}}} - m_{\mathrm{H}} + \frac{K_w}{m_{\mathrm{H}}}.$$

as a further approximation, pH is taken as $-\log m_{\mathrm{H}}$, an expression for β readily obtained by the differentiation of **10**:

$$= \frac{db}{dp\mathrm{H}} \approx -2.3026 m_{\mathrm{H}} \frac{db}{dm_{\mathrm{H}}} = 2.3026 \left[\frac{K_{bh} M m_{\mathrm{H}}}{(K_{bh} + m_{\mathrm{H}})^2} + m_{\mathrm{H}} + m_{\mathrm{OH}} \right] \quad (\mathbf{11})$$

Equation **11** permits some interesting observations regarding the nature of buffer value to be made. First of all, it may be noted that K_{bh} and m_{H} ely differ by more than 10-fold in the buffered range. At the point of maxi- um buffer capacity, as already noted, these two quantities are equal. Fur- ermore, between pH 3 and pH 11 (which includes the useful range of most ine buffers) the quantities m_{H} and m_{OH} are negligible in comparison with the

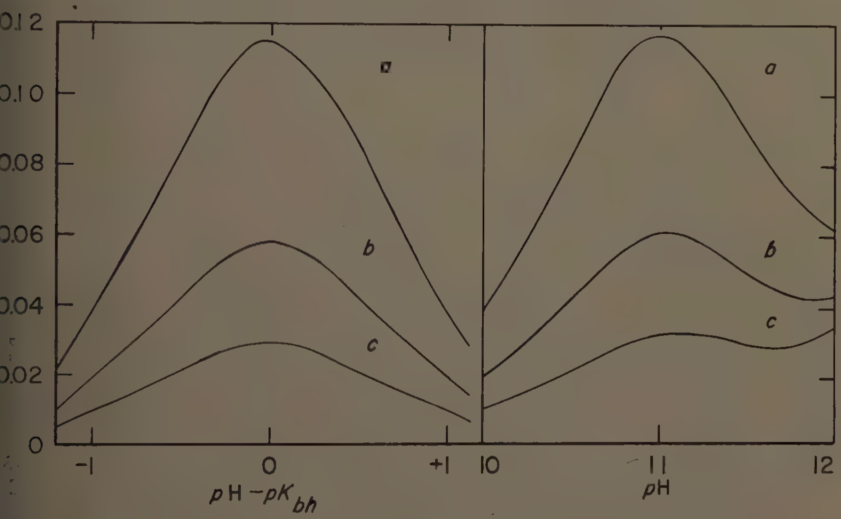


FIGURE 2. Buffer value of amine buffer solutions in the pH range from $pK_{bh} - 1$ to $_{bh} + 1$. Total concentration (amine + salt) = 0.2 (curve a), 0.1 (curve b), and 0.05 rve c).
The curves at the left apply when pK_{bh} lies between 4 and 10. The curves at right, com- ed for $pK_{bh} = 11$, illustrate the increased buffer capacity caused by the appreciable con- tration of hydroxide ion above pH 11.5.

t term within the brackets of **11**. Under these conditions, the largest value t β can assume is accordingly

$$\beta_{\max} = \frac{2.3026M}{4} = 0.576M. \quad (\mathbf{12})$$

t is evident that the buffer value can assume a larger value than that given **12** if the solution is strongly acidic or strongly alkaline and either m_{H} or $_{\mathrm{H}}$ is appreciable (see Equation **11**). Nevertheless, in practice these condi- ns are rarely encountered with the majority of amine buffer solutions. eridine and a few other amine bases are, however, sufficiently strong that $_{\mathrm{H}}$ contributes appreciably to the buffer value of the corresponding amine- buffer solutions.

Buffer values of amine-salt buffer systems are plotted in FIGURE 2 as a function of pH $- pK_{bh}$. The several curves represent the buffer values of so tions of different total amine concentrations. It should be emphasized that calculation of the buffer value as outlined here, in view of the approximati made, can be considered valid only for relatively dilute solutions.

Dilution Value

The buffer value alone is an insufficient index of the effectiveness o buffer solution in regulating the pH. The pH values of the most useful buff are not only relatively unaffected by the addition of small amounts of ac or bases but also insensitive to changes in the total concentration of the bu substances. Changes of the latter type may result from dilution or evapo tion of the solution. The "dilution effect," like the buffer capacity, can expressed quantitatively and in a uniform manner.[38]

The "dilution value," termed $\Delta pH_{1/2}$, has been defined as follows:

$$\Delta pH_{1/2} \equiv (pH)_{c_i/2} - (pH)_{c_i} .$$

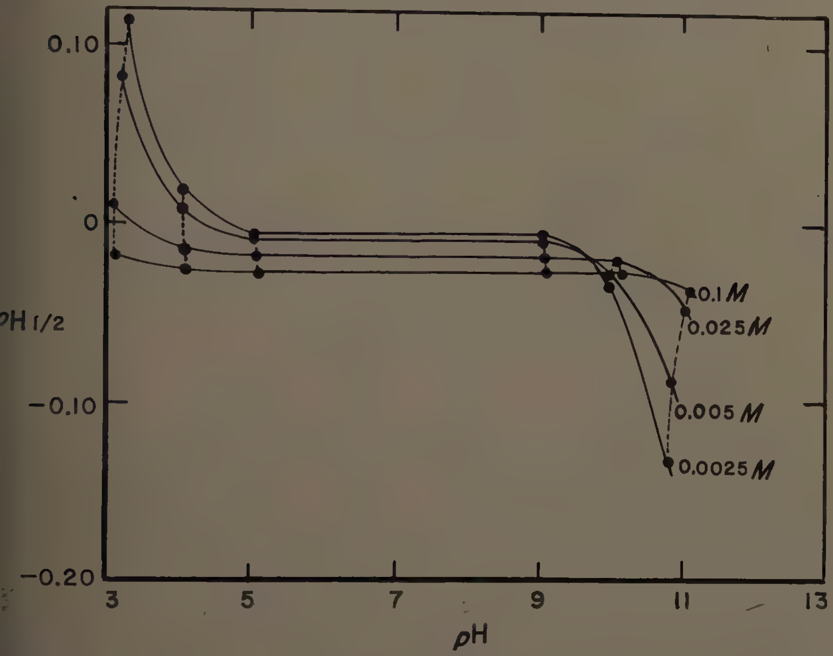
It is, therefore, the change of pH sustained by a solution of initial concent tion c_i upon dilution with an equal volume of pure water. The dilution va can, of course, be determined experimentally and, furthermore, can be cal lated approximately from theoretical considerations. The derivation is ba on a formulation of the acid-base equilibrium together with the Debye-Hüc equation in much the same manner as the computation of the buffer value

The complete equations for the dilution value of the buffer solutions various charge types are to be found in an earlier paper[38] and will not be peated here. The dilution value of amine buffer solutions consisting of eq molar concentrations of monoacid amine (B) and its conjugate ammonium (BH^+) varies with the molal concentration (m) of each species and with magnitude of pK_{bh} in the manner shown in FIGURE 3.

The dilution value of all but the most dilute equimolal amine buffers is ne tive at pH values greater than 4, which means that dilution causes a decre in the pH. Furthermore, the dilution value becomes less negative, shift toward positive values as the concentration decreases; the ultimate result that dilution increases the pH of dilute buffers composed of very weak ba (pH <4). There is also an inversion of the curves near pH 10. Above t point, the dilution value becomes more negative as the concentration decrea

It is perhaps surprising to learn that pure water of pH 7 often lowers the of an equimolal amine salt buffer even when the latter has a pH less than The explanation of this behavior is found in the two separate, and sometim opposing, effects caused by dilution. The first is the influence of the wa (which is both an acid and a base) on the buffer equilibria, tending to sh the pH toward the neutral point. The second is the effect of dilution on activity coefficients. The changes in interionic forces on dilution of the so tion tend to increase the activity coefficients and to decrease the pH of bu solutions of the type BH^+, B. The net result of these two effects is to cause pH of equimolal buffer solutions of this type to pass through a minimum if base is so weak that the pH is less than 7.

When the pH of the buffer solution lies between 4.5 and 9.5, the acid-b character of the water added is of negligible influence at moderate buffer c trations. In this region, the dilution value is determined solely by the nges in activity coefficients and does not depend on the dissociation constant the amine or its conjugate acid. Calculated dilution values[38] for equimolal ffer solutions of pH between 4.5 and 9.5 are given in TABLE 3 for several



IGURE 3. Dilution values of buffer solutions composed of equal molar amounts of amine and the conjugate acid (BH^+). The molar concentration of each species is indicated at right. Adapted from Bates[38].

TABLE 3

DILUTION VALUES ($\Delta p\mathrm{H}_{1/2}$) FOR EQUIMOLAL AMINE BUFFER* SOLUTIONS BETWEEN pH 4.5 AND 9.5, IN pH UNITS†

m	$\Delta p\mathrm{H}_{1/2}$	m	$\Delta p\mathrm{H}_{1/2}$
0.0025	−0.007	0.025	−0.019
0.005	−0.010	0.05	−0.023
0.01	−0.013	0.1	−0.028
0.02	−0.017		

Buffer: $BH^+(m)$, $B(m)$.
Data from Bates.[38]

es of m, the molality of the amine (B) and of the conjugate ammonium (BH^+).

Temperature Coefficient of pH

may be seen in Equation **5** that the pH is a function of three quantities, ely, pK_{bh}, the buffer ratio, and the activity-coefficient ratio. The buffer ratio is largely determined by the analytical composition of the solution. the intermediate pH range, pH 3 to 11, the buffer components are not appre bly hydrolyzed and, hence the buffer ratio is insensitive to the variation in activity of the water solvent with temperature. Furthermore, the change log f with temperature is determined by the magnitude of the partial mo heat content of the ionized species, that is, the amine salt in the solution.

It has been shown[39] that $d \log f_{\pm}/dT$ at constant pressure ($f_{\pm}$ is the m ionic activity coefficient) for four strong 1:1 electrolytes at 25° C. varies fr −0.0001 to −0.0003 at an ionic strength of 0.1. One may conclude, theref that the contribution of the last term of **5** to the temperature coefficient of for an amine buffer solution is very small.

The change of pH suffered by an amine buffer upon change of temperat is therefore largely determined by the magnitude of the change of pK_{bh} w temperature. The temperature coefficient of pK_{bh} at constant pressure is turn characterized by the heat of ionization of the acid BH^+, as shown by van't Hoff equation:

$$\frac{\partial pK_{bh}}{\partial T} = \frac{-\Delta H^\circ}{2.3026RT^2}.$$

The values of ΔH° for ammonium and substituted ammonium ions are su marized in TABLE 2. It will be noted that all these values are positive and in the range 8 to 14 kcal. mole^{-1}. Inasmuch as $2.3026RT^2$ has the va +406.8 kcal. mole^{-1} at 25° C., it is evident that (1) the pH of amine buf falls with rising temperature, and (2) the temperature coefficient of pH for these buffer systems, between −0.02 and −0.034 pH units/°C. T rather large change of pH with temperature constitutes one of the most seri disadvantages in the use of amine buffers for pH control.

Nevertheless the heat of ionization changes somewhat with temperat consequently the temperature coefficient of the pH of the buffer may be so what more favorable at one temperature than at another. The change pK_{bh} with temperature, which to a first approximation is also the change of buffer pH with temperature, is plotted in FIGURE 4 for several common ca acids, BH^+. The approximate values of $dp\mathrm{H}/dT$ (in pH units per degree 25° C.) for several useful amine buffer solutions and amino acid buffer soluti are summarized in TABLE 4. The values given are to be regarded as valid w the concentration of each buffer species is less than 0.1 mole/l.

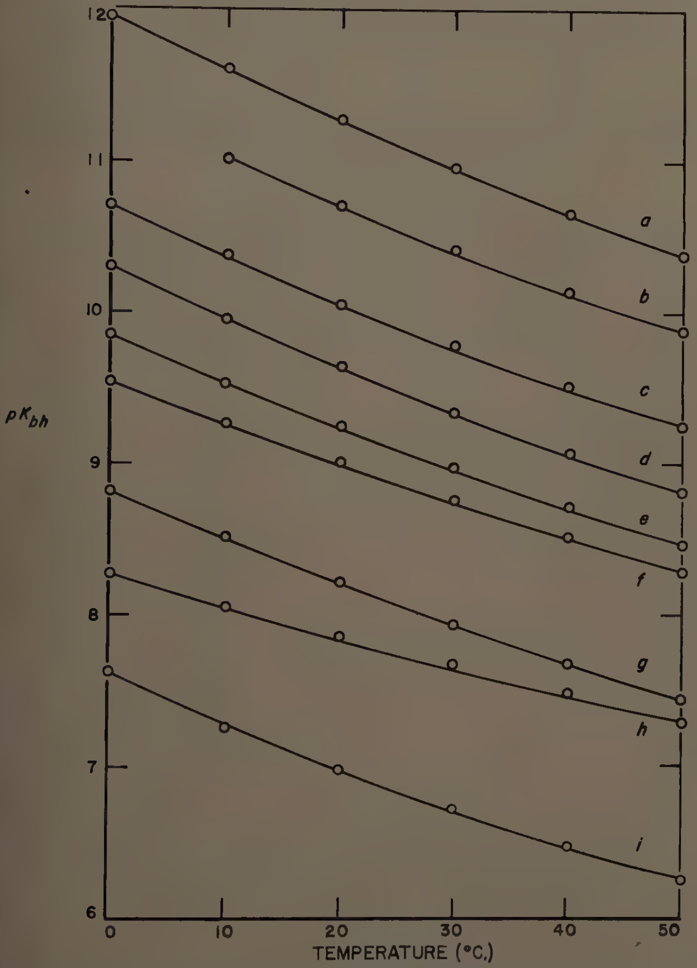
The pH of buffer solutions composed of an uncharged weak acid and salt (type HA, A^-) often passes through a minimum between 0° and 60 The minimum evidently occurs at the temperature where ΔH° becomes and changes its sign. Unfortunately, the heats of dissociation of cation a are of such magnitude that the minimum is usually displaced to temperat much higher than the normal working range.

Salt Effects

In terms of Equation **5**, the effect of adding a salt devoid of acidic or b properties to the buffer solution may be of two kinds. The first, which may called the specific salt effect, is not predictable. It results from a spe chemical interaction of the cation or anion of the salt with one of the bu

ies, altering the buffer ratio. An example is the well-known complexation
tion between cupric ion and ammonia or amines.

he second, usually called the neutral salt effect, results from the lowering
he activity coefficients of the ionized buffer species by the increase in the



GURE 4. Change of pK_{bh} with temperature. Key: *a*, piperidinium; *b*, γ-aminobutyric
c, ethylenediammonium (second step); *d*, monoethanolammonium; *e*, 4-aminopyridin-
f, diethanolammonium; *g*, tris(hydroxymethyl)aminomethane; *h*, triethanolammonium;
ylenediammonium (first step).

strength brought about by adding neutral salt. This effect is a very
ral one and, to the degree that the Debye-Hückel equation is valid, its
and magnitude are both predictable.

e user of amine buffers should always be aware of the possibility of chemi-
teractions that may alter the pH of the buffer medium. Special caution

should be exercised when heavy metal salts are added to the solution. specific salt effect, is something to be avoided rather than corrected.

Depending, as it does, on a change of the last term of Equation **5** with crease of ionic strength, the neutral salt effect may be considered as a sor "reverse dilution" effect. Indeed, it has been shown that the neutral effects determined experimentally for buffer solutions not far from the neu *p*H are of very much the same magnitude as the comparable dilution effe but of opposite sign.[39]

The change in *p*H is evidently determined by the change in $-\log (f_{BH^+}$ resulting from the increased interionic forces in the solution after additio the neutral salt. The Debye-Hückel formula (Equation **6**) indicates that

TABLE 4

TEMPERATURE COEFFICIENT OF *p*H FOR SOME AMINE BUFFER SOLUTIONS AND AMINO ACID BUFFER SOLUTIONS AT 25° C.

Amine (amino acid)	*p*H range	dpH$/dT$ (*p*H units/degre
Amine buffer solutions:		
Ethylenediamine	6–8	−0.027
Triethanolamine	6.8–8.8	−0.020
Tris(hydroxymethyl)aminomethane	7–9	−0.028
2-Amino-2-methyl-1,3-propanediol	7.8–9.8	−0.029
Diethanolamine	7.9–9.9	−0.025
4-Aminopyridine	8.1–10.1	−0.028
Ammonia	8.2–10.2	−0.031
Ethanolamine	8.5–10.5	−0.030
2-Amino-2-methyl-1-propanol	8.7–10.7	−0.032
Ethylenediamine	8.9–10.9	−0.029
Hexamethylenediamine	9–12	−0.034
Piperidine	10.1–12.1	−0.031
Amino acid buffer solutions:		
Glycylglycine	7.2–9.2	−0.026
Serine	8.2–10.2	−0.025
Glycine	8.8–10.8	−0.026
α-Alanine	8.8–10.8	−0.027
β-Alanine	9.2–11.2	−0.028
γ-Aminobutyric acid	9.5–11.5	−0.030

a first approximation, the activity coefficient of the uncharged amine (z_i = is unity both before and after the addition of salt and that an increase of ionic strength at low and moderate concentrations causes the activity co cients of charged species to decrease. The *p*H is accordingly raised, in di contrast to the salt effect on buffer solutions composed of an uncharged v acid and its salt, where addition of neutral salt brings about a decrease of

The magnitude of the neutral salt effect is, of course, dependent upon concentrations of the buffer species. Some typical salt effects, compute the Debye-Hückel equation, are given in TABLE 5.

Amine Buffers of a Definite Ionic Strength

Buffer solutions of varying *p*H but constant ionic strength are usefu kinetic and equilibrium studies as well as in physiological investigations.

acid amines are well suited for the preparation of buffer solutions of this , as has been shown elsewhere.[39]

stock solution of a salt of the amine, for example, the hydrochloride B·HCl, epared with added neutral salt. The concentration of the amine salt need nly great enough to give the desired buffer capacity, and the amount of ral salt should be sufficient to yield the desired ionic strength. The solu- of sodium hydroxide that is added to increase the *p*H will dilute the solu-

TABLE 5

APPROXIMATE SALT EFFECTS (IN *p*H UNITS) FOR AMINE BUFFERS* AT 25° C.

m	Salt effect for ΔI equal to†			
	0.01	0.02	0.05	0.1
0.01	+0.016	+0.027	+0.049	+0.070
0.02	+0.011	+0.020	+0.039	+0.058
0.05	+0.006	+0.012	+0.024	+0.039
0.1	+0.003	+0.007	+0.015	+0.025

uffer: BH^+ (m), B(m); *p*H 3 to 11.

Iolality of added salt, m_s, is given by $m_s = \Delta I$ (uni-univalent salts such as NaCl,), $m_5 = \Delta I/3$ (ternary salts such as Na_2SO_4 or $Ba(NO_3)_2$).

TABLE 6

NE BUFFERS OF CONSTANT IONIC STRENGTH: COMPOSITIONS OF STOCK SOLUTIONS

onic strength	Solution of amine salt		Solution of alkali	
	B·HCl (M)	NaCl (M)	NaOH (M)	NaCl (M)
0.05	0.02	0.03	0.1	0.05
0.1	0.02	0.08	0.1	0.1
0.15	0.02	0.13	0.1	0.15
0.2	0.02	0.18	0.1	0.2
0.25	0.02	0.23	0.1	0.25
0.05	0.05	—	0.2	0.05
0.1	0.05	0.05	0.2	0.1
0.15	0.05	0.1	0.2	0.15
0.2	0.05	0.15	0.2	0.2
0.25	0.05	0.2	0.2	0.25

nd lower the ionic strength unless sufficient neutral salt is added to this nt exactly to compensate for the dilution.

en this is properly done, the *p*H of the buffer solution may be shifted ghout the entire useful range of the particular buffer system without ng the ionic strength appreciably. The only restriction is that the num- moles of sodium hydroxide added should not exceed the amount of amine resent. Representative compositions of amine hydrochloride stock solu- and of the alkaline reagents needed for the preparation of buffer solutions e different ionic strengths are given in TABLE 6.

Preparation and Stability

One of the major drawbacks to the use of amine buffers for *p*H contr the difficulty of obtaining amines in pure form. Furthermore, few amines solids at room temperature. Most of the useful ones are liquids, and thos low molecular weight have high volatility; ammonia and the methylami are indeed gases at ordinary temperatures. It goes without saying that preparation of a solution of predetermined concentration by the weighing pure liquid, even of low volatility, is exceedingly difficult. In these ca one must usually resort to the use of a standard solution of the amine, concentration of which has been established by titration with standard a At best, this procedure is inconvenient and time-consuming. The *p*H at end point of the titration (final concentration = 0.05 *M*) is listed in TABL

Of the amines that have been discussed in the foregoing pages, only (hydroxymethyl)aminomethane, imidazole, and 4-aminopyridine are s substances commercially available in a state of adequate purity. These th buffer systems are able to cover th *p*H range 6 to 10 satisfactorily. In a tion, 2-amino-2-methyl-1,3-propanediol, 2-amino-2-methyl-1-propanol, h methylenediamine, and some of the substituted imidazoles, all solids at r temperature, are available commercially but must be subjected to fur purification before they can be used.

Some of the liquid amines are difficult to purify by distillation. Thi particularly true of the ethanolamines, which are liquids with very high boi points. Triethanolamine, whose mixtures with hydrochloric acid are part larly useful buffer solutions for the physiological *p*H range, is especially diffi to free from residual traces of monoethanolamine and diethanolamine.[7]

Triethanolamine hydrochloride and ethylenediamine dihydrochloride be obtained, however, in reasonably pure form. Amines indeed can be puri advantageously through the preparation of their hydrochlorides, with regen tion of the pure amine if necessary. The unpurified amine is dissolved suitable solvent (for example, benzene), and the insoluble hydrochlorid precipitated by passing dry gaseous hydrogen chloride into the solution. product is purified further by crystallization from a suitable solvent mix such as benzene and ethanol. The amine buffer solution is then prepared f a weighed quantity of the hydrochloride and a standard solution of sod hydroxide.

Several of the amino acids and substituted amino acids are suitable for bu uses, and these are available in a state of adequate purity. These amphol have, in general, a charge distribution that may be represented by $^+NH_3$— COO^-, or $BH^\pm$. The dissociation process is, then,

$$BH^\pm = B^- + H^+.$$

The dissociation constant, K_{bh}, for the cation acid group is sometimes refe to as the "second dissociation constant" inasmuch as this group is a wea acid than the carboxyl. The values of pK_{bh} and $\Delta H°$ for some of these stances are summarized in TABLE 7. The data have been collected by Robi and Stokes,[25] and the original sources are indicated in the last column of TA 7. In general, the buffer solutions are conveniently prepared from the a acid and a standard solution of sodium hydroxide.

he pH range, buffer value, and temperature coefficient of pH for amino buffers in the region where pH is regulated by pK_{bh} can be computed as ribed in the foregoing sections. The manner in which the activity coeffi- t of the dipolar ion changes with ionic strength is, however, not well under- d. Consequently, the calculation of the salt effect and dilution value ld probably not be attempted.

rom the standpoint of stability, amine buffer solutions are subject to the e limitations as all alkaline solutions, namely, such factors as changes of due to contamination with carbon dioxide from the air or attack of glass ainers. In addition, precautions must be taken to avoid loss of certain he amines of low molecular weight by volatilization from the solution.

TABLE 7

CONSTANTS FOR THE DISSOCIATION OF THE CATION ACID GROUPS OF AMINO ACIDS AT 25° C.

Amino acid	pK_{bh}	$\Delta H°$ (kcal. mole^{-1})	Reference
ylglycine	8.252	10.53	40
ne	9.208	10.35	41
roxyproline	9.662	9.35	41
ine	9.744	10.82	42
ine	9.780	10.55	43, 44
nine	9.866	10.98	42
rtic acid	10.002	9.04	40
nine	10.237	11.23	45
inobutyric acid	10.556	12.07	46

general, the pH of a 0.01 M solution of a monoacid amine is about 2 units er than pK_{bh}. Consequently, solutions of all but the very weakest amines absorb carbon dioxide from the atmosphere. In spite of statements to contrary,[16,17] the composition of dilute aqueous solutions of tris(hydroxy- yl)aminomethane is altered through the absorption of carbon dioxide from ir.

mine buffers are of considerable utility in pH control. Caution must be cised, however, in their selection, preparation, and preservation.

Summary

e important elements in characterizing the effectiveness of an amine buffer H control are: (1) stability, reproducibility and ease of preparation; (2) ange; (3) buffer value; (4) dilution value; (5) change of pH with tempera- ; and (6) salt effects.

e properties of buffer solutions consisting of an amine base (B) and its ugate acid (BH^+) in moderately dilute aqueous solution are readily pre- d (in first approximation) from a knowledge of the equilibrium behavior e weak acid, BH^+. The information needed includes the dissociation ant or pK_{bh}, the heat of dissociation, and some information on the activity icients of the buffer species.

e useful pH range is fixed in large part by the magnitude of pK_{bh}, extend- rom $pK_{bh} - 1$ to $pK_{bh} + 1$. The most important factor controlling the change of *p*H with temperature is the temperature coefficient of pK_{bh}, wh is in turn characterized by the value of the heat of dissociation.

Likewise, in the *p*H range 3 to 11 the Van Slyke buffer value is determi almost wholly by pK_{bh} and the concentration. The neutral salt effect, as as the change of *p*H on dilution of the buffer solution, can be predicted w the aid of the Debye-Hückel equation, which is a satisfactory representat of the activity coefficients in moderately dilute solutions.

The properties of amine buffer solutions useful for *p*H control are compa and summarized. Methods for the preparation of buffer solutions of differ *p*H at a fixed ionic strength are described.

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AMINE BUFFERS AS ACIDIMETRIC STANDARDS

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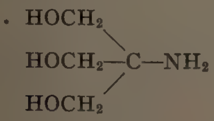
'he unusual biochemical properties of tris(hydroxymethyl)aminomethane he subject of this symposium. This compound was proposed as an acidi- tric standard by Fossum *et al.*[1] in 1951.

'here is some confusion concerning the multiplicity of names used by erent authors. The following explanation is offered to clarify the nomen- :ure situation: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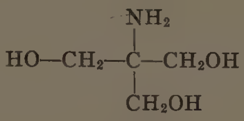
II

$$HO{-}CH_2{-}\overset{\displaystyle NH_2}{\underset{\displaystyle CH_2OH}{C}}{-}CH_2OH$$

I) Tris(hydroxymethyl)aminomethane was the term used by the firm* that :oduced the compound, to stress its functionality.

II) 2-Amino-2-hydroxymethyl-1,3-propanediol is the name used by *Chemical :tracts.*† The variation of 2-amino-2-hydroxymethylpropane-1,3-diol seems)e preferred by the British.

'ris, Trisamine, Trisamino, abbreviations of I, are suitable only for notes tabulation headings if properly identified. THAM, or Tham, has been d in the literature: it is the name for one brand‡ of purified tris(hydroxy- :hyl)aminomethane.

'romethamine is the generic name§ for this compound.

Summary

'he ideal requirements for any analytical chemistry standard are rigid. : requirements differ slightly depending on their use.

:xperimental evidence and data are presented to evaluate tris(hydroxy- :hyl)aminomethane as an acidimetric standard. It is concluded that the ne is a good, but not ideal, standard. Tris(hydroxymethyl)aminomethane the following properties that are important considerations for evaluation n acidimetric standard:

l) Tris has a dissociation constant in water[2] of 1.202 at 25° C. (compare $\times\ 10^{-6}$ for potassium hydrogen phthalate). It does not meet the ideal iirement in water. The inflections at the equivalent point of the poten- ıetric titrations curves in water with hydrochloric acid and in acetic acid ı perchloric acid are sharp. Its inflection in acetic acid is about the same ;nitude as sodium acetate, one of the stronger bases in this solvent. There- it is near ideal for acetic acid.

The Commercial Solvents Corporation, New York, N. Y.
The Chemical Abstracts Service, The Ohio State University, Columbus, Ohio.
Fisher Scientific Co., Pittsburg, Pa.
Used by the Abbott Laboratories, North Chicago, Ill.

(2) Tris has a good equivalent weight, 121.136. Its equivalent weigh less than that of potassium acid phthalate, 204.22, and greater than sodi carbonate, 53.00. If the optimum volume of 0.1 N solution to be sta ardized (30 to 40 ml.) is used, it will require 0.4 gm. of tris. The maxim weighing error on a balance precise to ±0.1 mg. would be ±0.14 mg., whic of the same order as the error for reading the buret.

(3) Tris is a crystalline solid of definite composition.

(4) It can be prepared in a high state of purity.

(5) It is stable under conditions of preparation and use. There are lim tions on heating to remove moisture.

(6) It is stable for long periods of storage, at least 12 years.

(7) It is not hygroscopic at usual laboratory humidities. Its critical hum ity point is above 90 per cent relative humidity at 25° C.

(8) There is no evidence that Tris absorbs carbon dioxide from the ai the solid state or in aqueous solution in amounts that will interfere under ditions of titration.

(9) Tris is sufficiently soluble in a number of solvents to be used as an a metric standard for nonaqueous solutions.

(10) It is available commercially at a reasonable cost.

There is no known ideal acidimetric standard. The rapid expansion of a base titrations to include all solvents[3] has expanded the ideal requirement include solubility in a wide variety of solvents. One cannot consider the sta ard's properties only in water. Part of the material from the original propo will be included in this paper.

EQUIPMENT AND REAGENTS

Equipment

Buret, volumetric, 50 ml., calibrated by the National Bureau of Standa Washington, D.C.

Titration apparatus and *p*H meter* and dual titrometer.†

Reagents

Hydrochloric acid, 0.1 N, prepared from ACS Reagent grade hydroch acid.

Sodium hydroxide, 0.1 N, prepared from a 50 per cent aqueous ACS Rea grade sodium hydroxide solution in freshly boiled water.

Perchloric acid, 0.1 N, in acetic acid, prepared from ACS Reagent g materials according to directions given by Markunas and Riddick.[4]

Tris(hydroxymethyl)aminomethane, purification described by Fossum *et*

Potassium hydrogen phthalate, National Bureau of Standards sample effective neutralizing power 100.05 per cent and Mallinckrodt's primary sta ard grade, neutralizing power assumed to be 100.00 per cent.

Sodium carbonate, purified by method of Waldbauer *et al.*[5]

4-(4-Dimethylamino-1-naphthylazo)-3-methoxybenzenesulfonic acid i r solution (DNM), Eastman organic chemicals No. 1954, previously listed p-sulfo-o-methoxybenzeneazodimethyl-1-naphthylamine (SMN[1]). Titu-100 mg. of the indicator in a mortar with 2.59 ml. of 0.1 *N* sodium hy-ide solution and dilute to 100 ml. with boiled water.

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* No. 7661-A1, Leeds and Northrup Co., New York, N. Y.

† Precision Scientific Company, Chicago, Ill.

hyl orange indicator solution. Dissolve 100 mg. of the indicator in 100 ooiled water.

ethyl red indicator solution. Dissolve 100 mg. of the sodium salt in 100 of boiled water.

ixed indicator. Mix equal parts of 100 mg. bromocresol green triturated mortar with 1.45 of 0.1 *N* sodium hydroxide solution and dilute to 100 vith boiled water, and 100 mg. of alizarin red S (sodium alizarin sulfonate) lved in 100 ml. of boiled water.

eference buffer solution, *p*H 4.70, for colorimetric determination of the point. Mix 50 ml. of 0.1 *N* potassium hydrogen phthalate, 13.1 ml. of V sodium hydroxide solution, 3 drops of the appropriate indicator solution ops of the mixed indicator) and 25 ml. of boiled water.

ater. Demineralized or distilled water distilled from alkaline permanga-in all-Pyrex-brand glass still with an efficient spray trap. Discard first last 10 per cent.

COMPARISON OF STANDARDS

is(hydroxymethyl)aminomethane was compared with two commonly standards,[6] sodium hydroxide solution and sodium carbonate, by stand-ing a hydrochloric acid solution with each.

e sodium hydroxide solution was standardized potentiometrically with ssium hydrogen phthalate, National Bureau of Standards standard sample The hydrochloric acid solution was standardized with the standard m hydroxide solution potentiometrically, and with sodium carbonate rding to the method of Kolthoff and Sandell.[7]

e standardization with tris(hydroxymethyl)aminomethane was carried by the following procedure: approximately 0.5 gm. of the amine was ac-ely weighed into a 250-ml. tall-form beaker. The sample was dissolved ml. of recently boiled water and titrated potentiometrically with the ochloric acid solution. The results of standardization are given in TABLE 1.

e *p*H at the equivalence point of tris(hydroxymethyl)aminomethane and ochloric acid under the conditions of standardization was determined ematically and graphically to be 4.70. The neutralization curve of the e and hydrochloric acid shows a clean sharp break at the equivalence point. standard solutions were corrected for change in volume due to tempera-change.

SELECTION OF INDICATORS

veral indicators whose *p*H range included the equivalence point of the ion were selected for test. The indicators were screened by adding a few of their solution to a solution of the amine, and titrating potentio-cally with hydrochloric acid. Three indicators, 4-(4-dimethylamino-1-thylazo)-3-methoxybenzenesulfonic acid, ethyl orange, and a mixed indicator consisting of equal parts of 0.1 per cent solutions of bromocresol g and sodium alizarin sulfonate, gave a distinctive color at a *p*H of 4.70.

The three indicators selected from the screening tests were evaluate follows:

Tris(hydroxymethyl)aminomethane (0.5 gm.) was accurately weighed a 250-ml. Erlenmeyer flask and dissolved in 50 ml. of recently boiled dist water. Three drops of the indicator solution (6 drops of the mixed indic were added, and the amine was titrated with standard hydrochloric acid.

TABLE 1

STANDARDIZATION OF SOLUTIONS

Standard:	$HKC_8H_4O_4$	NaOH	Na_2CO_3	$(CH_2OH)_3C$
Solution standardized:	Sodium hydroxide, *N*	Hydrochloric acid, *N*		
	0.10127	0.10075	0.10093	0.1008
	0.10126	0.10075	0.10084	0.1007
	0.10129	0.10068	0.10084	0.1007
	0.10130	0.10071	0.10088	0.1007
	0.10131	0.10071	0.10093	0.1007
	0.10131		0.10088	
Mean	0.10129	0.10072	0.10088	0.1007
Mean dev.	0.000017	0.00002	0.00003	0.0000

TABLE 2

PURITY OF TRIS(HYDROXYMETHYL)AMINOMETHANE AS DETERMINED WITH SEVERAL INDICATORS

	Potentiometrically* (%)	DNM† indicator (%)	Ethyl orange indicator (%)	Mixed indic (%)
	99.92	99.95	99.98	99.96
	100.00	99.92	100.04	99.93
	99.99	99.97	99.94	99.93
	99.96	100.00	100.02	
	99.95	99.91	99.97	
Mean	99.96	99.95	99.99	99.94

* Calculated from data in TABLE 1.

† 4-(4-Dimethylamino-1-naphthylazo)-3-methoxybenzenesulfonic acid.

end point of the titration was determined by matching with the color o reference buffer solution. The strength of the standard acid was 0.1007 (TABLE 1). The results given in TABLE 2 were obtained.

THERMAL STABILITY

The stability of tris(hydroxymethyl)aminomethane was determined at and at elevated temperatures.

Three portions of the amine were stored in a desiccator for three days phosphorus pentoxide and weighed each day. No change in weight was tected.

Portions of the amine were heated in a laboratory oven at 103° C., remo l, and periodically weighed. Typical data are given in TABLE 3, sample 1. ger sample was treated in the same manner except that portions periodi- were taken for analysis (Sample 4, TABLE 3).

plicate titrations were run on 0- and 10-hour portions of Sample 4. The y remained unchanged. The loss in weight shown by Sample 1 may be o volatilization; however, there is no other evidence that indicates that ydroxymethyl)aminomethane volatilizes at 103° C.

ample was heated at 110° C. for 10 hours, weighed, and analyzed. It was t cream color speckled with brown spots at the end of the heating period. nalysis showed definite decomposition (TABLE 4).

TABLE 3

STABILITY OF TRIS(HYDROXYMETHYL)AMINOMETHANE AT 103° C.

mple No.	Heating time, hours	Weight loss		Analysis (%)
		(mg.)	(%)	
1	2	0.8	0.040	—
0150 gm.	4	1.5	0.074	—
	6	2.3	0.114	—
	8	2.5	0.124	—
	10	3.2	0.159	—
4	0	—	—	99.95
	2	—	—	99.95
	4	—	—	99.99
	6	—	—	99.94
	8	—	—	99.98
	10	—	—	99.94

TABLE 4

STABILITY OF TRIS(HYDROXYMETHYL)AMINOMETHANE AT 110° C.

Heating period (hours)	Weight of sample (gm.)	Analysis (%)
0	2.0409	99.95
10	2.0237	98.82

data in TABLE 3 indicate that the amine may be dried for 2 to 4 hours at 103° C. It is considered safer to dry, when necessary, in a vacuum t 60° C. at 10 mm. of mercury pressure or less, for 2 hours. This obviates e of a thermometer that may be in error.

HYGROSCOPICITY

hygroscopicities were determined for tris(hydroxymethyl)aminomethane everal materials commonly used as standards. The sample of tris- xymethyl)aminomethane was the same material used for the titrimetric s.

linckrodt's primary standard grade potassium hydrogen phthalate and Reagent grade potassium chloride were recrystallized twice from conductivity-type water. The salts were dried, ground to pass a 50-mesh scr redried in an oven overnight at 105° C., and stored in a vacuum desiccator phosphorus pentoxide in vacuo until ready for use.

Mallinckrodt's primary standard grade benzoic acid was recrystallized tw from neutral 95 per cent ethyl alcohol which had recently been fraction distilled. It was dried, ground to pass a 50-mesh screen, redried, and st in a vacuum desiccator over phosphorus pentoxide in vacuo until ready to

The hygroscopicities were determined at 25° ± 0.5° C. in desiccators taining a salt in contact with its saturated solution as described in the In national Critical Tables.[8] Representative data are shown in TABLE 5 for gain in weight at the end of the 10-day period.

The hygroscopicity tests were carried out in May, a period during which air temperature and humidity varied widely. The adsorbed moisture on weighing bottles is the cause of the major error in the low adsorption rang

TABLE 5
HYGROSCOPICITY OF SEVERAL STANDARDS AT 25° C.

Relative humidity	Gain in weight (%)			
	Tris(hydroxymethyl)aminomethane	Potassium hydrogen phthalate	Benzoic acid	Potassium chl
31%	0.20	0.15	0.03	0.04
51	0.18	0.20	0.03	0.06
71.2	0.18	0.20	0.05	0.09
87	0.20	0.24	0.01	0.24
91	0.24	0.20	0.01	0.64
96.6	38.3	0.34	0.04	126.3
100	45.8	0.36	0.58	138.3

The hygroscopicity of tris(hydroxymethyl)aminomethane compares fa ably with that of the common primary standards which were tested ove humidity ranges that might be encountered in laboratory work.

STANDARD FOR NONAQUEOUS TITRANTS

Potassium acid phthalate was proposed as a standard for perchloric aci acetic acid by Seaman and Allen[9] and used by Markunas and Riddick[4] at al the same time. It is good standard for acids in nonaqueous solvents. H ever, it has two distinct disadvantages. The base strength has not evaluated. It can not be used directly for the standardization of aqu acids.

Often it is informative if a base is analyzed in both water and a nonaqu solvent. A standard that can be used for both solutions is preferred so error of varying base equivalency is eliminated. Tris(hydroxymethyl)am methane may be used for either aqueous or nonaqueous standardization. is slowly soluble in acetic acid. It can not be heated to speed solution or acetic acid partially acetylates the amine group.

PURITY OF COMMERCIALLY AVAILABLE HIGH PURITY TRIS(HYDROXYMETHYL)AMINOMETHANE

ιere are four suppliers of purified tris(hydroxymethyl)aminomethane. erial was obtained from each supplier and analyzed potentiometrically by ous and nonaqueous titration, in April and May, 1960.

allinckrodt's primary standard grade potassium hydrogen phthalate was eference standard. It was assumed to have an acid and base equivalent 0.00 per cent.

ıe aqueous hydrochloric acid was standardized as previously described. perchloric acid in acetic acid was standardized by the method of Markunas Riddick.[4] The tris(hydroxymethyl)aminomethane was dissolved in c acid by magnetic stirring. The results are given in TABLE 6.

TABLE 6
ANALYSIS OF HIGH PURITY TRIS(HYDROXYMETHYL)AMINOMETHANE

Supplier	Aqueous		Nonaqueous	
	Purity	No. of replicates	Purity	No. of replicates
CSC*	99.98%	6	100.04%	5
I	99.96	8	100.06	4
II	99.92	6	100.07	4
III†	99.88	5	100.02	4
III‡	99.90	4	100.12	8
IV†		16	99.78	5
IV‡	99.94	5	100.03	4

Average standard deviation 0.03_3

ame material reported in TABLE 2.
nalyzed as received; IV gave erratic results. Deviations from 99.32 to 99.79 per cent btained for 16 replicates.
inely ground.

aterials I and II were fine, uniform crystals. One bottle of II contained particles that may have been boiling chips. III had larger crystals and ill larger and varying in size. It had been observed in the original study large crystals analyzed low. It is believed that solvent is trapped in the r crystals.

e purity by nonaqueous titration is higher than by aqueous titration. phase of the study was undertaken only to compare the basicities in each nt, not to compare the basicities between solvents. Potassium hydrogen alate does not have acid and base equivalency of 100.00 as assumed. rally, it has been found that the acid equivalent is 100.01 to 100.05 per a few lots have been as low as 99.98 per cent. These values were obtained mparing to NBS Sample 84c. It is assumed that if the acid equivalent ater than 100, the base equivalent will be less than 100. There may be er explanation. It has been found that most amines give a higher value e base-enhancing solvent, acetic acid. This difference may be as large er cent. The acetic acid enhances the strength of the weaker base components that do not titrate in water, but will titrate in acetic acid. As amine becomes purer, the water and acetic acid values approach each othe

STABILITY ON STORAGE

The CSC material was prepared in 1948 and analyzed 99.96 per cent aga NBS Standard Sample 84c potassium hydrogen phthalate. It analyzed 9 per cent in 1960 against Mallinckrodt's primary standard grade potass hydrogen phthalate assuming the acid equivalent to be 100.00 per cent. T was no change during the 12 years. No special protection was given material. It sat in the laboratory reagent cabinet in a glass-stoppered bo with an aluminum foil cover most of the time. Within the past few year bulk had been reduced such that it was kept in the standards desiccator.

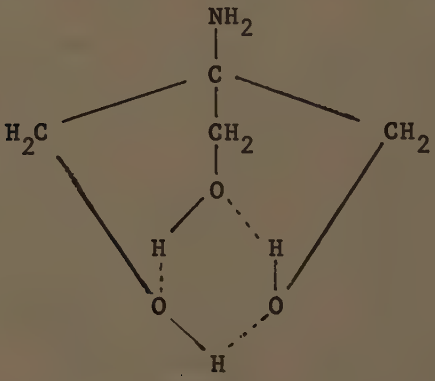

FIGURE 1.

ABSORPTION OF CARBON DIOXIDE

Specific tests for carbon dioxide absorption have not been made. The no evidence of the crystalline amine having absorbed carbon dioxide ov period of 12 years. Aqueous solutions of the amine have been made in afternoon in a titration beaker covered with a watch glass and titrated next morning. The titration curve did not show any evidence of car dioxide interference.

Possible Structure

Benesch and Benesch[10] proposed an intramolecular hydrogen bonding f infrared studies. The two dimensional structure is shown in FIGURE 1. T structure accounts for some of the unusual reactivity of tris(hydroxymeth aminomethane.

ACKNOWLEDGMENT

I thank D. L. Kiser for the excellent work that he did in comparing the commercial materials in both water and acetic acid.

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FORMATION OF MICROEMULSIONS BY AMINO ALKYL ALCOH

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Introduction

The use of emulsions in biological systems is becoming technically import for the study of the particulate fat absorption process in the intestine and p duction of chylmicrons for oil/water systems and for the delayed absorption proteins, such as allergens, from subcutaneous injections of water/oil emulsi In each case the emulsion-stabilizing agents must be compatible with the sorbing material and with biological systems involved. It has recently b shown[1,2] that certain amino alkyl alcohols are excellent agents for the pro tion of microemulsions and the study of the mechanism of formation of th emulsions by such compounds has now been undertaken.

Micro- and Macroemulsions

The fundamental difference between these two types of oil and water disp sions is that the equilibrium for the macroemulsions is for the ultimate sepa tion of the dispersion into two separate oil and water phases, whereas the eq librium for the microemulsions is for the production of a greater dispersion permanent separation of the two phases into droplets of either oil or wa Therefore macroemulsions seldom possess droplets of smaller diameter t 5000 Å, whereas the microemulsions are usually in the range of 100 to 60 diameter droplets. Since the macroemulsion droplet sizes are comparable w the wave length of light, they are characterized by strong light scatter whereas the microemulsions are transparent, optical isotropic, faintly lig scattering and obey the light-scattering laws applicable to large molecu The closely packed spheres of either oil or water give, consequently, crys clear optical systems. When the dispersion is in the shape of cylinders, bea ful anisotropic optical birefringent effects are observed on streaming.[4] If microemulsion is placed in a high gravity ultra centrifuge, the droplets can treated as molecules and their dimension can be calculated from their sedimen tion constants.[3] It is interesting that the ultracentrifugal sedimentation f does not break these microemulsions and that they remain stable. The ma emulsions would, on the other hand, instantly break with this treatment. I angle X-ray investigation of these transparent isotropic microemulsion syst shows a single diffuse scattering band which indicated the center to center tance between the droplets.[5] Recent work using biological staining[1,2] te niques with osmic tetroxide on the oil dispersions containing amphipathic m cules with double bonds, have enabled these droplets to be seen directly in electron microscope down to diameter dimensions of 75 Å in the isotr transparent systems.

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* J. B. Montagne holds a Petroleum Research Fund Fellowship from the American Ch cal Society, Washington, D.C.

The Physical Chemistry of Microemulsions

n investigation of the physical chemical criteria for the formation of micro-lsions, it has been shown by Bowcott and Schulman[3] that a concentration mphipathic agents is required greater than that concentration necessary to g the oil/water interfacial tension to zero. Macroemulsions, on the other d, form before the interfacial tension reaches zero. This indicates that the rfacial tension γ_i must have a metastable negative value, which would give gative free energy variation $-\gamma dA$ (γ = surface tension; A, surface area), ch could be used to break up the droplets and increase the surface area at oil/water interface after two or more drops have coalesced. This would ilize the disperse phase and prevent phase separation.

he total interfacial tension can be defined $\gamma_i = \gamma_{o/w} - \pi$, where $\gamma_{o/w}$ = the water interfacial tension without the addition of the amphipathic agents; the spreading pressure of the added amphipathic at the oil/water interface. hus if $\pi > \gamma_{o/w}$, there will be a negative interfacial tension γ_i and micro-lsions will form since there is free energy now available to increase the in-cial area. The equilibrium condition will be reached when $\pi = \gamma_{o/w}$ or at interfacial tension. If $\pi < \gamma_{o/w}$, macroemulsions will form and, since the rfacial tension is now positive, the surface area will diminish and the oil water phases will separate.

he direct measurement of the metastable negative interfacial tension at oil/water interfaces is impossible since the interface would immediately k up and spontaneously emulsify. However if a counter tension is placed he interfacial measuring device (for example a Wilhelmy plate) to support interface and prevent surface breakup, π can be directly calculated.

$$\gamma_i = \gamma_{o/w} - \pi = \gamma_{a/w} - \gamma_{o/a} - \pi_m \tag{1}$$

he right-hand term in the equation is that obtained by the pull on a Wil-ny plate at a duplex film interface; $\gamma_{a/w}$ = surface tension of the aqueous/air rface; $\gamma_{o/a}$ = surface tension of the oil/air interface; π_m = surface pressure sured. Except for π all the terms in the above equation are now all experi-tally determinable; this then enables π and γ_i to be calculated.

uplex films considered here consist of a mixed monolayer of amphipathic ecules penetrated by the oil phase molecules. These are formed by mono-r penetration of the surface adsorbed interfacial monolayer of one amphi-ic compound, such as a fatty acid or an alkyl alcohol by another surface ve agent in the aqueous phase. This produces, as is known,[6] high surface sures, π. This mixed film is now again penetrated by nonpolar oil mole-s, which have been mixed into the system. Since the ratio of oil molecules ne amphipathic molecules is made high (50:1), on compression of the mixed on a Langmuir trough, some of the oil molecules will be incorporated in the ed film and others will be squeezed out and will spread on the mixed mono-r in the form of a very thin oil film (150 to 500 Å thick). This spreading be visually seen by color interference patterns as the compression of the ed monolayer proceeds. The tension now at this oil/air interface $\gamma_{o/a}$ holds interface together and permits π_m to be measured, although π is greater than $\gamma_{o/w}$. The thickness of the duplex film is approximately equal to t diameter of the oil droplets formed by the microemulsion produced by the age used to form the interfacial structure (FIGURE 2).

Mechanism of Formation of Microemulsions

π-Surface pressures can be obtained spontaneously by monolayer penetrati of an alkyl alcohol or cholesterol monolayer by ionic surface active agents su as salts of alkyl sulphate and alkyl amines and substituted amines injected in the underlying solution, at constant area of the insoluble monolayer. Th π-surface pressures at the air/water interface can reach to values of more th 60 dynes/cm.,[6] although the collapse π pressure for the single surface compone monolayers on their own are below 35 dynes/cm. If the surface pressure held constant and below the 60 dyne/cm. but above the 35 dyne/cm., imm diate expansion of the interfacial area takes place as the molecules of ionic su factant penetrate the monolayer at the air/water interface.

The analogous penetration of the mixed film by the oil molecules at the o water interface to increase the surface area is the basis for the formation of t microemulsions. However the penetrating pressure in this case must be grea than the oil/water surface tension $\gamma_{o/w}$, which is always less than the $\gamma_{a/w}$ te sion of 72 dynes and is approximately 50 dynes/cm. for hydrocarbon and dynes/cm. for aromatic hydrocarbon compounds. Therefore it is progressiv easier to obtain negative interfacial tensions for these systems, provided th the oil molecules can penetrate the interfacial mixed film. Microemulsio made by these concepts have been prepared and details published.[1,2,3]

The Role of Amino Alkyl Alcohols in Microemulsion Formation

Very recently[1,2] a somewhat different type of mixed film has been prepar that enables the oil molecules to penetrate the interfacial mixed film. This obtained by spacing the fatty acid molecules on the structural lattice of an a sorbing large counterion (FIGURE 2), thus permitting the oil molecules to fo readily a mixed film with the fatty acid molecules. Microemulsions imme ately form on addition of 2-amino-2-methylpropanol to fatty acids in the pr ence of hexadecane (cetane) or kerosene at *p*H 8.8 or also with the addition an alkyl alcohol to the fatty acid molecules when the *p*H is greater than 10.5 a the chain length of the alcohol is greater than the paraffin molecule cha length.[1]

The structure of the interfacial film at the oil/water interface and the me urement of the π_m surface pressure can be investigated under identical con tions to those producing the microemulsion.

If the stearic acid is spread with paraffin molecules at ratio of 1 fatty a molecule to 50 paraffin molecules on the Langmuir trough and surface pressu is measured with a sand-blasted platinum plate* (2.5 cm.) at *p*H 2 (HCl), t fatty acid π/A curve is identical with or without the hexadecane. The layer over the fatty acid film can be seen as a myriad of little droplets with interference colors, and the surface pressure develops at 24Å^2 per molecule.

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* H. Rosano, private communication.

f now the fatty acid and hexadecane is spread on NH$_4$Cl at *p*H 10.5, a mentary expansion of the film is observed which then goes quickly into solu- n at very low pressures. If Tris(tri-methanol-amine) is added instead of I$_4$OH no difference is observed, and the mixed oil fatty acid monolayer again lapses. It is interesting that under these conditions no microemulsions can formed (FIGURE 1, curve 2).

f now 2-amino-2-methyl-1-propanol (AMP) or 2-dimethylamine-2-methyl-1-panol (DMAMP), both of which form excellent microemulsions, are added the underlying solutions, and a fatty acid film is spread, values greater than

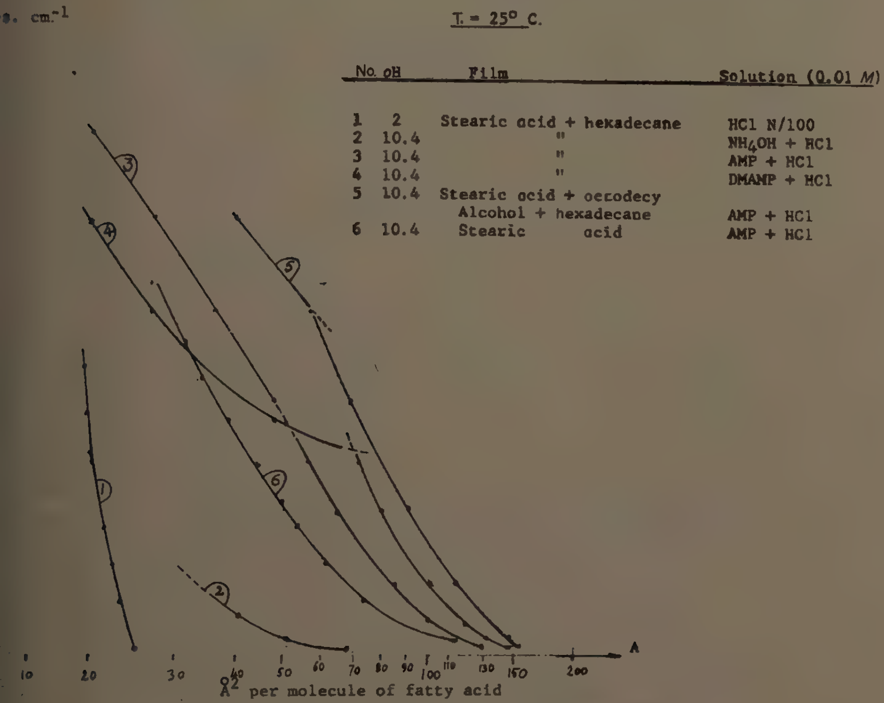


FIGURE 1. Structure of Duplex film.

Å^2 per fatty acid molecule at low pressures are obtained with or without the sence of hexadecanol at *p*H's around 10.5 (curve 3 and 4, FIGURE 1 respec- ely). In the presence of hexadecanol (curve 5) more stable pressures and her areas per fatty acid molecule (up to 150 Å^2) are obtained. The stability the π/A curves (FIGURE 1) definitely reflect the order of microemulsion bility.

)n compression of the mixed films, large surface pressures develop up to 55 nes/cm. The penetration of the hexadecane molecules into the monolayer be seen by the development of interference colors and definite spreading of oil on the AMP stearate monolayer. No droplets or lenses appear, as with matic oils. Different oils, aromatic or alkyl, behave quite differently, but se effects will not be discussed in this paper. The large areas of the mixed film can be produced only by adlineation of the amine propanol molecule the negative ionic group of the fatty acid and the interlocking of further am propanol molecules by hydrogen bonding of the orientated array of hydro groups (FIGURE 2). These films alone without the oil molecules also colla only at high surface pressures (curve 6). These films, of course, will not stabilized by the opposing surface tension of the oil/air interface, $\gamma_{o/a}$, as the case of the duplex films. If AMP is injected under a stearic acid film constant area 20 Å, no penetration or rise in surface pressure takes place less nonpolar oil molecules are added to make a duplex film (curves 1 and

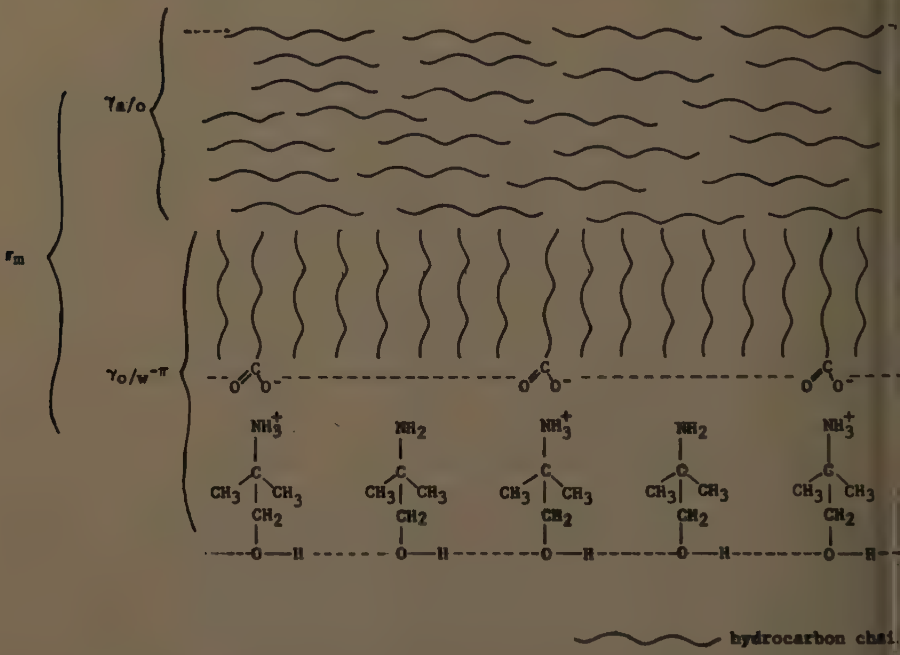
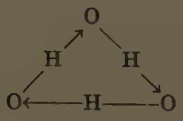


FIGURE 2. Force/area curves of Duplex film.

Discussion

It may be seen from the above-described results that conditions for formation of microemulsions and expansion of the fatty acid monolayer o supporting lattice occur with the penetration of the oil molecules into this op lattice structure. These structures support high metastable surface pressu and are produced by the hydrogen bonding between the alcohol groups attac to the amino alkyl compound. If three alcohol OH groups are attached to single amino group such as tris, no open-lattice structure is formed, and



groups are now hydrogen bonded *intra*molecularly[7] and the molecule be-
es like the ammonium cation. It is interesting that this parallels the bio-
cal activity of the amino compounds. Tris or triethanol amino compounds
biologically compatible, whereas the mono- or dihydroxy compounds readily
n microemulsions with fatty acids and are biologically active and form *inter*-
ecular complexes.

f we now apply Equation 1 to the results obtained in FIGURE 1, it may be
ı that the concept of negative interfacial tension can be expressed in experi-
ıtal terms. It may be seen from the equation that the negative interfacial
sion term can be readily measured if the opposing surface tension of the
, oil/air surface tension, is kept as high as possible. Hexadecane is con-
iently high at 29 dynes/cm. at 25° C. and, by insertion into Equation 1, one
ains for a π_m surface pressure of 55 dynes/cm. measured under metastable
ditions in FIGURE 1 a negative interfacial tension of -12 dynes/cm.

$$\gamma_i = \gamma_{o/w} - \pi = \gamma_{a/w} - \gamma_{o/a} - \pi_m$$

$$\gamma_i = 50 - \pi = 72 - 29 - 55 = -12$$

$_{/w)}$ is then equal to 62 dynes/cm.).

Summary

he mechanism of formation of microemulsions with amino alkyl alcohols
been discussed. This has been described in terms of the structure of the
rfacial mixed film produced by the adlineation of the hydroxyl groups in
amino alkyl alcohol when they adsorb as counterions to the fatty acid mole-
s in the monolayer.

his adlineation permits *inter*molecular hydrogen bonding, if the amino alkyl
hol has one or two hydroxyl groups per molecule, and this bonding forms
interfacial lattice structure. The trihydroxy compounds (Tris) form *intra*-
ecular hydrogen bonding and the molecules behave as ammonia. These
pounds form no interfacial structures and no microemulsions.

he interfacial spreading pressures π produced by these open-lattice struc-
s in the presence of the hydrocarbon molecules produce the negative free
gy variation necessary to stabilize the microemulsions as permanent dis-
ions, with the greatest interfacial area. The interfacial spreading pressure
the case of macroemulsions is smaller than the oil/water interfacial tension
, and thus the free energy term, in equilibrium, tends towards the smallest
rfacial area and separation of the emulsion into two phases. A method of
suring the negative interfacial tension term when $\pi > \gamma_{o/w}$ has been given
he use of a counter tension at the oil/air interface $\gamma_{o/a}$, when duplex films
used on the Langmuir trough.

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ON THE USE OF CARBONIC ANHYDRASE IN CARBONATE AN AMINE BUFFERS FOR CO_2 EXCHANGE IN MANOMETRIC VE SELS, ATOMIC SUBMARINES, AND INDUSTRIAL CO_2 SCRUBBER

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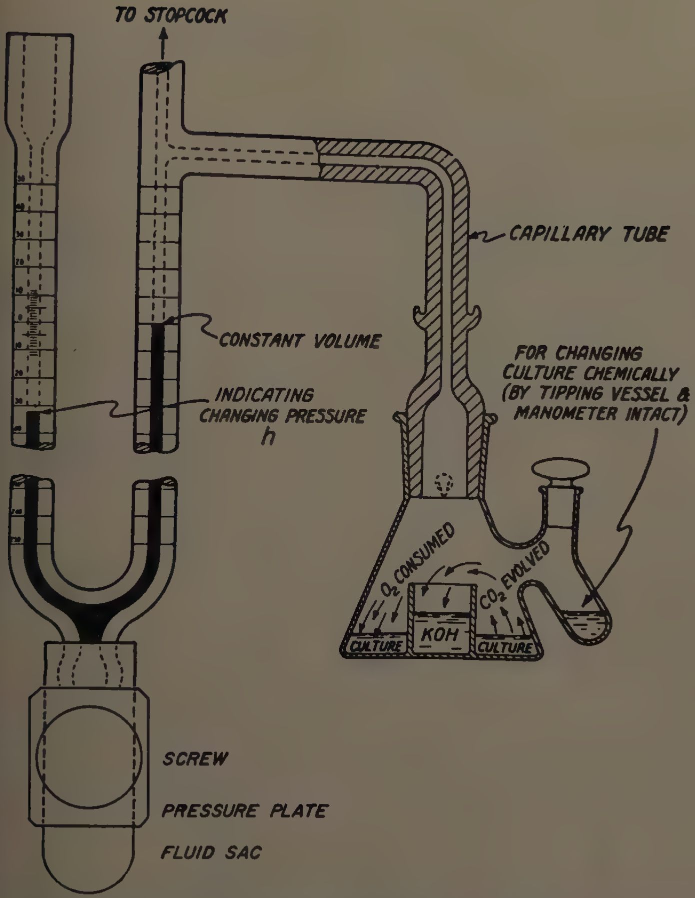
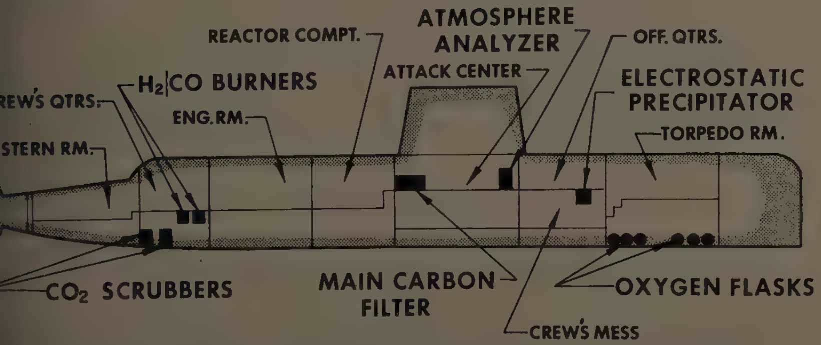
There is a remarkable resemblance between the kinetics of carbon diox gas exchange in manometric vessels of 1 cu. inch volume (FIGURE 1)[1] and atomic submarines of 100,000 cu. ft. volume (FIGURE 2),[2] and it is not the p pose of this paper to hide such a kinetic similarity. As will be indicated some detail, the over-all mathematical treatment of CO_2 in the gas phase much the same whether one is dealing with CO_2 absorption in (1) a manomet vessel central well (FIGURE 1) containing, for example, KOH, NaOH, LiC K_2CO_3-$KHCO_3$, Tris buffer (THAM), or mono- or di-ethanolamine; or in an atomic submarine CO_2 scrubber containing monoethanolamine (FIGURE and 4) or LiOH (FIGURE 5). The weaker alkaline CO_2 buffers mentioned (c bonate, Tris, and the ethanolamines) have all been found to undergo tremend acceleration of CO_2 exchange when the enzyme carbonic anhydrase is added my hands accelerations in rate of 5- to 100-fold have been readily obser with all such buffers, depending upon conditions. Strong alkalis ($pH > 11$ 12) destroy the enzyme rapidly, of course.

The manifold acceleration of CO_2 exchange by carbonic anhydrase may observed with respect to either absorption or elimination of CO_2 gas, depe ing upon the side of the equilibrium point from which one is operating; can be a variable function of, for example, the *p*H, base exchange, or tempe ture. In this connection it is worth recalling well-known physiological sit tions in which different *p*H-base equilibria are involved at constant (bo temperature. In the stomach mucosal wall and in kidney cells, carbonic hydrase accelerates the removal of CO_2 from the blood, leading to increa acidity in the stomach lumen and urine; in the lungs and pancreas, on the ot hand, carbonic anhydrase accelerates the removal of CO_2 gas from the blo leading to more alkaline blood and pancreatic juice. Analogously, in a s marine CO_2 scrubber operated at two different temperatures, carbonic an drase could accelerate the absorption of CO_2 by the lean buffer mixture in low-temperature absorber and, when this mixture has become enriched w CO_2 and raised to an appropriately higher temperature in the stripper, carbo anhydrase could accelerate CO_2 stripping, provided that the absorbing and stripping operations were carried out somewhere between 0° and 60° C., practical temperature limits for stable action by this enzyme. Parenthetica it may be remarked that the submarine CO_2 scrubber provides an "ex breather" for the crew in addition to their lungs. Various engineering po bilities for feasibility studies in connection with CO_2 elimination from ato submarine atmospheres will be considered in the latter part of this paper. us look at manometric situations first.



GURE 1. Typical Warburg manometer and illustrative vessel[1] for operation at constant e of gas space; x (cu. mm. NTP) gas change = hk, where h = mm. pressure change 0 mm. Brodie fluid = 1 atmosphere), and k = $([V_G \cdot 273/T] + V_L\alpha)/P_0$, V_G = volume phase (cu. mm.), V_L = volume of liquid phase (cu. mm.), P_0 is a pressure of one at- here expressed in millimeters of confining fluid, T is the absolute experimental temper- and α is the Bunsen absorption coefficient of the gas exchanged.



URE 2. Atmosphere control equipment on a typical nuclear-powered submarine, with reference to indicated size and location of CO_2 scrubbers.[2]

Manometric Measurements

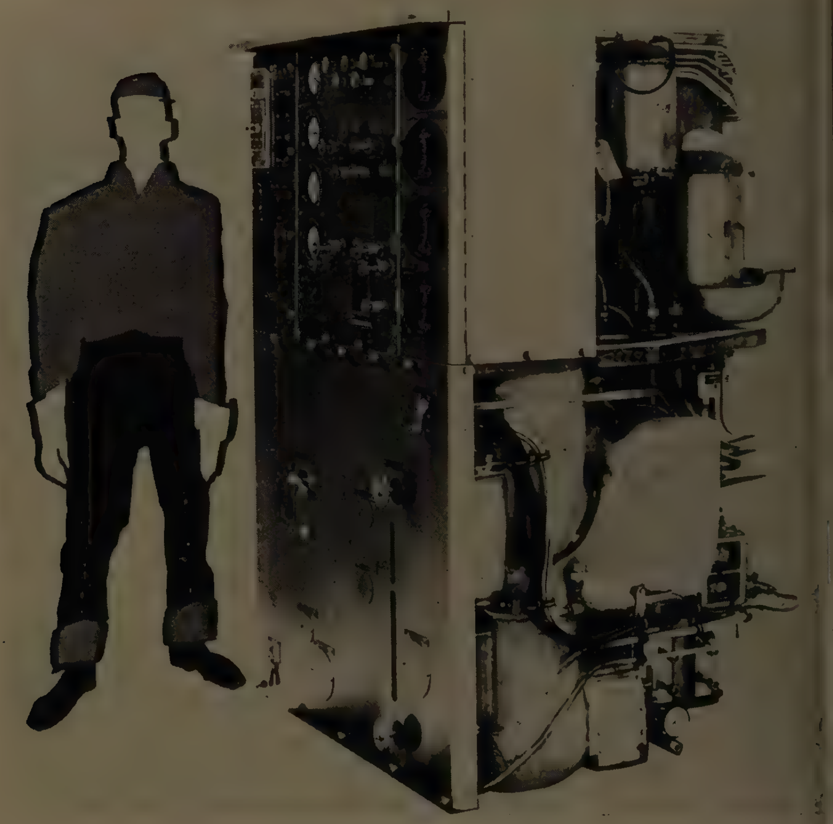
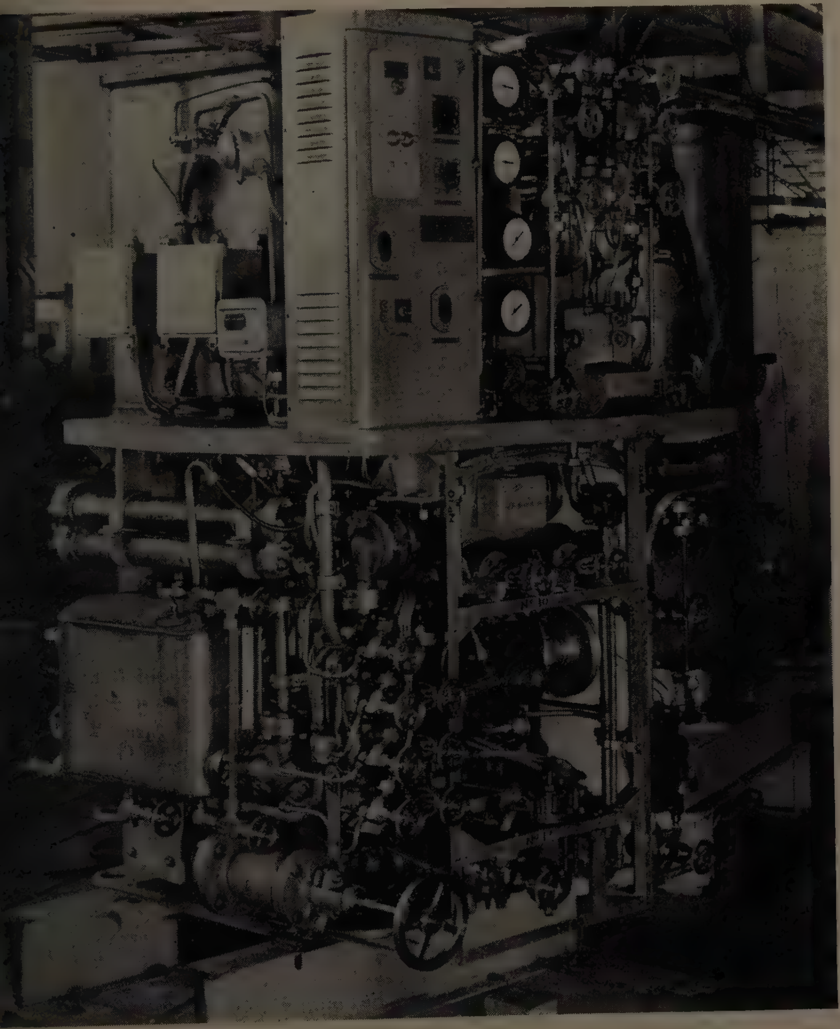


FIGURE 3. Submarine CO_2 removal plant, front and left-side view, at man-sized sc

The advantages and disadvantages of various ethanolamines as CO_2 buff and absorbents in manometric measurements were pretty well outlined abo 10 years ago by Pardee,[3] Krebs,[4,5] and Burk *et al.*[6] Among such amines manometry, diethanolamine was found to be the most generally suitable w respect to desired K_b value, solubility, nonvolatility, CO_2 retention, and ma tenance of constant CO_2 gas pressure up to the order of 2 to 3 per cent-atm phere. The chief disadvantages of even the best of the amines tested w residual autoxidation that could not be entirely eliminated, even with 0.1 cent thiourea; difficulty in satisfactorily attaining a physiological CO_2 par pressure of about 5 per cent in the gas phase at about 1 atm. total pressure; rates of absorption considerably lower than those shown by strong alkalis (s as KOH and NaOH) in small absorption containers.

The most interesting observation that can be made about the manome use of ethanolamine CO_2 absorbents at present is that quite recently it has b d that they are equaled or surpassed in virtually all aspects by concentrated ssium carbonate-bicarbonate buffers containing carbonic anhydrase (War- and Krippahl,[7] and Stambuk and Burk[8]). The carbonate-bicarbonate



IGURE 4. Submarine CO_2 scrubber, exposed view front and right side. Hardware ier than CO_2-absorbents occupies most of the space.[2]

fers can be adjusted readily to provide fixed CO_2 pressures over a several usandfold range from less than 0.01 per cent-atm. up to well above 10 per t-atm., with high CO_2 retention, no autoxidation, and a satisfactory equilibra- time of but a few minutes at most, providing that suitable manometric sels are employed, notably and preferably the new vessels described by Warburg and Krippahl.[9] In such vessels, illustrated in FIGURE 6, the small cent well on the floor of the main compartment (of the vessel shown in FIGURE 1) replaced by an elevated wide-area trough that is optionally further connect directly with a side arm; indeed there may be an additional independent si arm.[9] For high physiological CO_2 pressures of the order of 5 per cent-atm., t carbonate-bicarbonate buffers will be of the order of 1 to 3 *M* (and largely

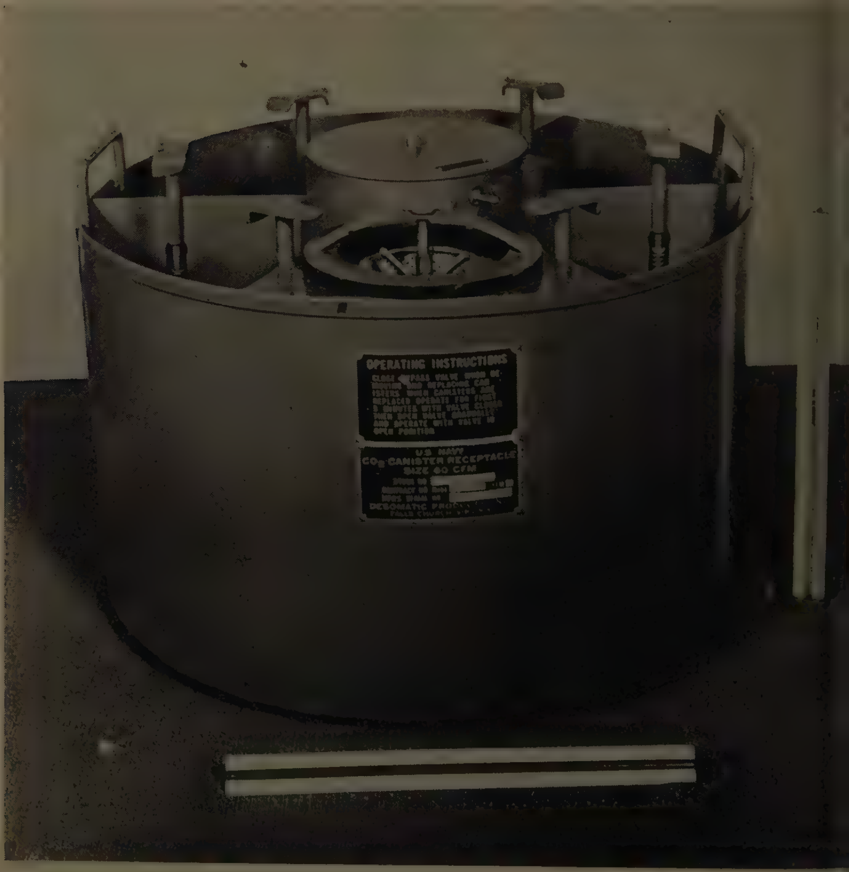
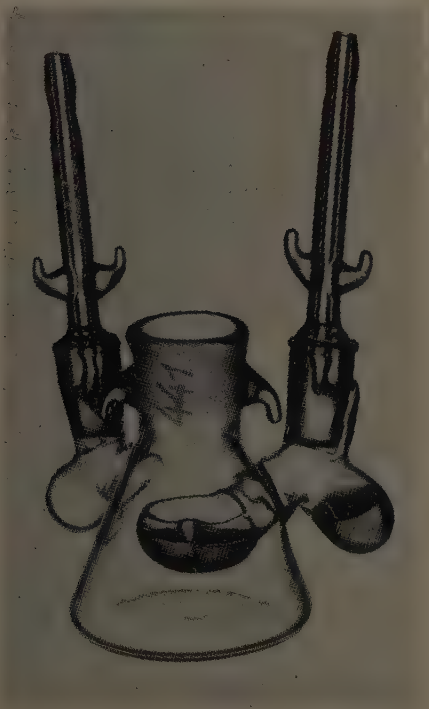


FIGURE 5. CO_2-canister with six LiOH units capacity.[2]

carbonate), and will contain 5 to 10 Meldrum and Roughton units[10] of carbo anhydrase per cc. (that is, about 0.001 mg. highly purified enzyme/cc., or up 1 mg. commercially prepared enzyme/cc., depending upon the purity of t latter). The vessels with a trough to contain buffer also improve the usa of the amine buffers by similarly decreasing the equilibration time, as a res of increased area and volume of buffer that may be employed conveniently a given vessel.

The new manometric vessels, carbonate-bicarbonate buffers, and carbo ydrase thus provide, at long last, a *one*-vessel method for measuring at con- nt and *physiological* CO_2 gas pressure either the oxygen consumption or oxy- production by animal or plant cells or tissues suspended in a medium and of choice, and in a medium physically separated from the buffer mixture; er such conditions, therefore, the manometers may then register pressure nges solely due to oxygen gas exchange uncomplicated by simultaneous bon dioxide gas exchanges, at least under steady-state conditions that we l now consider in some detail. Here it is important to distinguish between



IGURE 6. Manometer vessel with central elevated trough and confluent side-arm.[9]

quite different kinds of CO_2 steady-state pressures, one determined chiefly uffer *equilibria* and the other determined chiefly by the *rate* of CO_2 absorp- in the CO_2-absorbing liquid. The latter case will be considered first; for it, on and Elliott[11] give a somewhat similar kinetic treatment and derivation offers a useful comparison with that given here, but their results are ex- sed in terms of quantities (volumes) of CO_2 rather than of pressures of CO_2 are of prime interest here.

se 1. *Absorption rate steady-states.* The steady-state pressure of CO_2 in vessel gas phase, $pCO_{2\infty}$, will here be a function of the difference between he rate of production of CO_2 by, for example, cellular material in the liquid ium in the main compartment of the vessel; and (2) the rate of absorption O_2 in the central well or trough containing either strong alkalis (KOH, NaOH) or relatively alkaline amines or carbonates (low proportion of bic bonate). Indeed $p\mathrm{CO}_{2\infty}$ can be shown to be simply RV_L/a, where R is the r of production of CO_2 expressed as cu. mm. normal temperature and press (NTP)/unit t/cc. liquid medium in the main compartment, V_L is the num of such cc. in the compartment, t is time, and a is the specific absorption r constant whose numerical and algebraic values (see below) depend upon fact such as the exposed cross-section area of the buffer in the well or trough, kind and concentration of CO_2 absorbent in the well or trough, the rate shaking, the temperature, and the geometry of the vessel. However, a can determined empirically, for any given set of conditions and vessel to be ployed, by observing the change in pressure with time when CO_2 gas, which been liberated suddenly from a medium containing bicarbonate (or carbona by addition of excess HCl or other strong acid from a vessel side arm, is sorbed by alkali or buffer in the well or trough; when the natural logarithm the pressure is plotted against time, a linear (first order) curve is obtained wh slope is a/k, from which a may be obtained by elimination of k, the ordin (and ordinarily predetermined) Warburg vessel constant expressed in desi units.* In order for the pressure of CO_2 to be expressed in the desired term per cent-atmospheres NTP (100 per cent = 760 mm. Hg = 10,000 mm. Bro fluid), k is calculated as [10] $[(273\ V_G/T + (V_L\ \alpha_{\mathrm{CO}_2})]$ sq. mm., where V the volume of gas space in the vessel in cc., T is the absolute temperature, α_{CO_2} is the Bunsen solubility coefficient of CO_2 in the liquid medium. The fac k, converts a CO_2 gas pressure change, h_{CO_2}, in per cent atmospheres (per ce atm.) into cu. mm. NTP CO_2 gas change in the vessel (see legend to FIG 1), and is here exactly 100 times the numerical value of the ordinary Warb vessel constant, k, that converts CO_2 gas pressure change in mm. Brodie f to cu. mm. NTP CO_2 gas change in the vessel.

At the start of any manometric experiment, the steady-state pressure of in the vessel gas phase is not established instantaneously, but will usu require some minutes or seconds for practical attainment, in accordance w the equation

$$d\ p\mathrm{CO}_2/dt = RV_L/k - (a/k)p\mathrm{CO}_2\,.$$

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* The rate of absorption of CO_2 in the well or trough can readily be shown experimen to be proportional at any moment to the pressure of CO_2 in the gas phase; that is,

$$-d\ p\mathrm{CO}_2/dt = (a/k)\ p\mathrm{CO}_2,$$

$$\int_{p\mathrm{CO}_2'}^{p\mathrm{CO}_2} d\ p\mathrm{CO}_2/p\mathrm{CO}_2 = (a/k)\int_{t}^{t'} dt,$$

and

$$\ln\ (p\mathrm{CO}_2/p\mathrm{CO}_2') = (a/k)\ t,$$

where $p\mathrm{CO}_2$ is the pressure observed at time t and $p\mathrm{CO}_2'$ is any subsequent pressure at sequent time t', and a/k is the slope of Equation **C**.

s equation yields, upon integration, and evaluation of the integration con- t by placing $pCO_2 = 0$ when $t = 0$,

$$pCO_2 = (RV_L/a)(1 - e^{-at/k}). \qquad (2)$$

ing $t = \infty$,

$$pCO_{2\infty} = RV_L/a \qquad (3)$$

is evident from Equation **3** that, once the necessary constants have been letermined for a given set of conditions, calculation of the steady-state sure of CO_2 in the vessel gas phase is very simple and exact, as are like- , from Equation **2**, the times required to attain particular fractions of the dy-state pressure. Thus the time required to attain 90 per cent of the dy-state value may be calculated by putting $pCO_2 = 0.9\ pCO_{2\infty}$ in Equa- **2**, whence

$$0.9\ (RV_L/a) = (RV_L/a)(1 - e^{-at/k}),$$

$$e^{-at/k} = 0.1,$$

$$-at/k = \ln 0.1,$$

hat

$$t_{90\%} = -(2.3 \log 0.1)/(k/a).$$

n ordinary manometric vessel similar to that illustrated in FIGURE 1, con- ing 2 N KOH in the well, k is usually about 150 mm.^{2} for a vessel of some c. volume, and a is commonly about 70 min.$^{-1}$, whence the time required ttaining 90 per cent $pCO_{2\infty}$ is about 5 min. and, for attaining 99 per cent $_{2\infty}$, about 10 min. (twice as long, precisely). It is to be noted that the time any fractional attainment of CO_2 steady-state pressure is independent of rate of CO_2 production, RV_L. On the other hand, a steady-state pressure O_2 in the vessel gas phase of 0.1 per cent-atm. (= 10 mm. Brodie fluid) ld occur, with a about 70 min.$^{-1}$, when $RV_L/a = 0.1$, or $RV_L = 7$ cu. mm. /min., or h = about 5 mm. pressure change of CO_2 per minute.

bviously, the steady-state pressure of CO_2 in the vessel gas phase can be ed by any of the ways in which R, V_L, and a can be varied, as best suits nvestigator with the particular apparatus he has at hand. In general, the dy-state pressure will be lower the more effective is the CO_2 absorption (the ter the value of a), the smaller the value of V_L, the smaller the concentra- of cellular matter, or the lower the rate of CO_2 production per unit of cel- material. With appropriate choice of a, V_L, and (especially) R, it is ily possible to attain and to maintain steady-state pressures of CO_2 as low 001 per cent-atm. (0.1 mm. Brodie fluid). R may be varied widely by em- ing widely varying concentrations or amounts of cellular material per ves- Such a methodology allows one to study the effect of very low concentra- of CO_2 upon various metabolic processes, such as the respiration or growth icroorganisms. Years ago, co-workers and I were able to show[12] that the

growth of a given strain of yeast, *Saccharomyces cerevisiae* strain Fleischma
139,[13] was still maximal at a CO_2 pressure of no greater than 0.001 per cent-a
when the cells were grown with biotin; when the cells were grown with the
aminocarboxylic (opened-ring) derivative of biotin (DAC), however, CO_2 pr
sures about 100 times as great were required; likewise, at 1 per cent-atm. C
the growth versus biotin concentration curve attained its half-maximum
maximum values at concentrations of biotin that were 100 times smaller th
those of DAC for the corresponding growth versus the DAC concentrat
curve and, at 0.001 per cent-atm. CO_2, this difference was increased anot
100-fold (10,000-fold altogether). Such data were part of our evidence at
time[14] that the function of biotin in living organisms was closely connected w
the CO_2 requiremnet, a view that has been fully substantiated since by ma
workers, especially by the recently added chemical evidence provided in vari
papers by Lynen and his co-workers.

In TABLE 1 is given illustrative, previously unpublished data taken by us
years ago on the effect of CO_2 pressure on the growth velocity* of the nitrog
fixing bacterium, *Azotobacter vinelandii*, that is an application of material
sented in the two previous paragraphs; here, since growth over a considera
period of time was involved (7 hours), there was some shifting in the stea
state pressures that required a certain amount of averaging with respect
time, but without notable distortion of essential end results and conclusi
It is to be noted that the growth was logarithmic, and that the velocity c
stants of growth were calculated from semilog plots of either respiration rat
turbidity with respect to time.

---

* It was established more than 30 years ago that a minimum pressure of carbon dioxi
essential for the normal growth of many, if not all, heterotrophic (as well as autotrophic)
ganisms. An absolute CO_2 requirement was shown to exist clearly distinguishable f
beneficial stimulation, utilization as a source of carbon, or buffering action in the exte
medium. Such findings were established by experimental methods that were qualitati
accurate but, for the most part, semiquantitative at best. In general, cultures were grow
the presence of CO_2-absorbing alkali without, however, the concentrations of CO_2 in ga
liquid (or solid) phases being adequately defined. Occasionally, streams of CO_2-free and
containing air or nitrogen were employed, but again with the same disadvantage.

Even when cultures were grown with large but confined gas volumes, the initial press
of CO_2 became altered during the course of the experiment by additional metabolic
formation; satisfactory corrections therefore were difficult to apply since the CO_2 req
ments of most organisms are satisfied at very low pressures of CO_2, usually below those exis
in air (0.03 per cent-atm.), and any small amounts of CO_2 that were produced created relati
large pressure changes with respect to CO_2 pressure requirements. Most of the experim
were carried out with stationary or slightly shaken cultures, with the result that it was
possible to determine, even by thermodynamic calculation, the relative or absolute amoun
CO_3^{--}, HCO_3^-, H_2CO_3, or CO_2 in the liquid phase from the CO_2 in the gas phase, even w
the latter was known approximately. This is an important consideration if it is desire
further determine which of these substances might be mechanistically responsible for the "
effect."

Although equilibrium between the three first-named forms is established almost ins
taneously, the formation of CO_2 from them, or vice versa, is relatively slow and of the s
general order of rate as the still further exchange of CO_2 between gas and liquid phase.

Finally, the growth function in the early experiments was not based on studies of
velocity constant of growth, but only upon the amount of growth; the latter, in contrast to
former, necessarily varies with the duration of the experiment. TABLE 1 represents a ty
experimentation in which many of these problems and difficulties are overcome in good m
ure. Space considerations and relative interest do not permit presentation here of co
erations of CO_3^{--}, HCO_3^-, and H_2CO_3 functions that may be derived from the experime
tation illustration in TABLE 1, but many interesting kinetic and thermodynamic deduct
could be drawn from such experimentation.

se 1 continued. Absorption rates with different types of manometric vessels CO_2 absorbents. Before considering Case 2 it will be desirable to give a historical sketch to date of actually observed rates of CO_2 absorption in ous types of manometric vessels in common use with the principal types of absorbents of interest here. The Barcroft manometric vessel illustrated in RE 7, developed in England many decades ago, is an example of a vessel

TABLE 1

THE VELOCITY CONSTANT OF GROWTH OF AZOTOBACTER AS A FUNCTION OF PRESSURE OF CO_2 IN THE GAS PHASE*

culture per vessel (cc.)	0.5	1.0	2.0	3.0	4.0
gas space per vessel (cc.)	17.01	18.06	18.48	17.98	17.98
ssel constant (mm.^{2})	153	162	166	161	161
ecific absorption constant (hr.$^{-1}$)	2700	2700	3350	3350	3350
, respiration rate per vessel (cu. n. O_2 or CO_2/hr.; R.Q. = 1)					
Initial (t = 0 hr.)	5.0	10.0	20.0	30.0	40.0
Final (t = 7 hr.)	9.0	26.0	64.0	102.0	144.0
Mean† (per hour)	6.7	16.0	36.0	56.0	76.0
(mean per cent-atm. CO_2 in s phase [RV_L/a])	0.0025	0.0059	0.0108	0.0168	0.0227
growth‡ (t = 7 hr.)					
Respiration rate (cu. mm. O_2/hr./cc.)	18	26	32	34	36
Relative turbidity (per cc.)	54	84	100	108	114
ean velocity constant of growth					
rom respiration rate	0.084	0.139	0.168	0.177	0.188
rom relative turbidity	0.076	0.139	0.165	0.176	0.188
verage	0.080	0.139	0.167	0.177	0.182

he material used was a 1-day-old *Azotobacter vinelandii* culture, at 31°C. temperature, 6.8, in an inorganic medium with 1 per cent glucose. The duration of the experiment hours. The source of nitrogen for growth was the N_2 gas in air; the vessel was of the illustrated in FIGURE 1, containing 0.3 cc. of 2 N NaOH in the central well. The CO_2 gas phase was supplied by that formed in respiration. The total pressure in the gas was 1 atm., and the rate of respiration per unit of cells was constant with time, so that crease in rate per vessel with time is a measure of the rate of growth and is accompanied corresponding increase in the number of organisms, turbidity, cell nitrogen, and other s. The manometers can be read as frequently as desired; hence, time curves of the se in growth may be determined over relatively short periods of time, such as hours or es.

These previously unpublished experiments were carried out with Hans Lineweaver and enneth Horner in 1930 at the Fixed Nitrogen Research Laboratory, United States rtment of Agriculture, Washington, D.C.

ntilog ([log final rate + log initial rate]/2). Since the increase in rate of respiration growth was logarithmic and not linear with respect to time, the true average rate is by the logarithmic mean and not by the arithmetic median; that is, not by (initial final rate)/2. The arithmetic median values are, however, only slightly higher than oarithmic mean values, namely, 6.75, 18, 42, 66, and 92 respectively.

The initial "growth" at t = 0 was, per cc. of culture: respiration rate, 10 cu. mm. O_2/hr.; e turbidity, 32.

he velocity constant of growth, g, is given by 2.3 (log final growth − log growth)/t, since growth is (and was) a logarithmic (first order) process, the velocity ich is proportional at any moment to the concentration of cells (that is, to respiration relative turbidity, and other factors) obtaining at that moment. In the present exents, the rates of respiration for the different vessels were measured every hour; upon g these rates semilogarithmically against time, straight lines were obtained that re- a very slight bending upward corresponding to the increases in partial pressure of the gas phase as the rates themselves increased (that is, as RV_L/a values increased). reatest relative increases took place where the increase in CO_2 pressure had the least in the range where the limiting maximum growth velocity was being approached.

that is "practically useless"[11] for rapid absorption of CO_2 even by strong al
in the central well *unless* filter paper wetted with the alkali is placed in the
(FIGURE 8). The area of the exposed liquid in the well without filter pap
too small in relation to the volume of gas space involved (about 35 cc.), so t
without the filter paper one-half hour or much more is required to absorb a
sonably small amount of CO_2 in the gas phase, with strong alkali as absorb
and, of course, longer with the CO_2 buffer absorbents. Likewise, as indic
in FIGURE 9, when a manometric measurement of respiration is taking pl

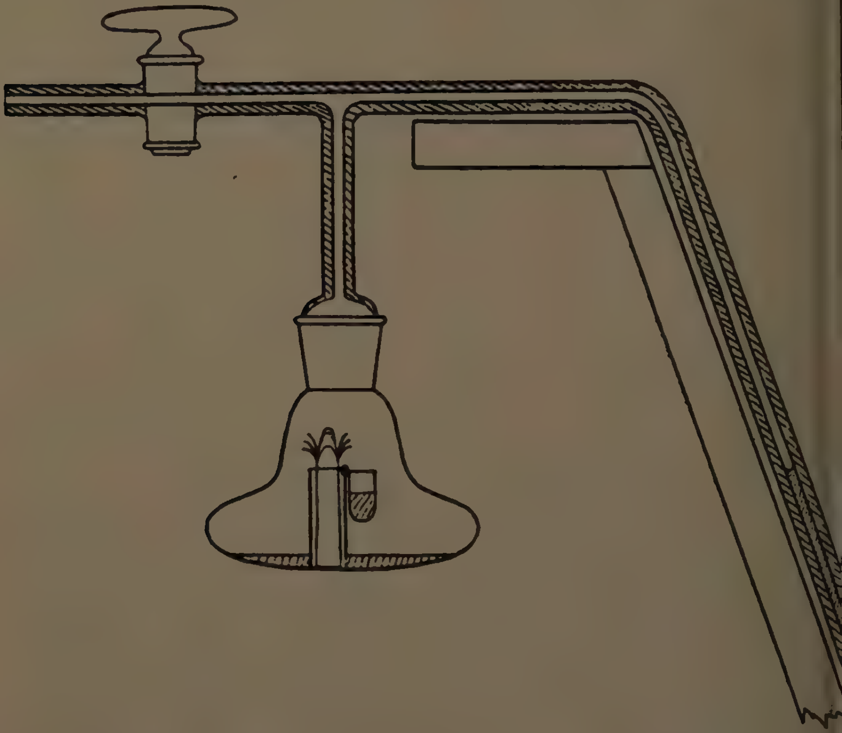
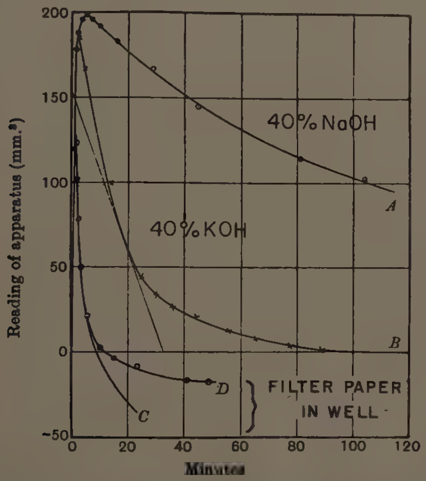


FIGURE 7. Side view of Barcroft manometric vessel attached to differential manomet
one side only shown.

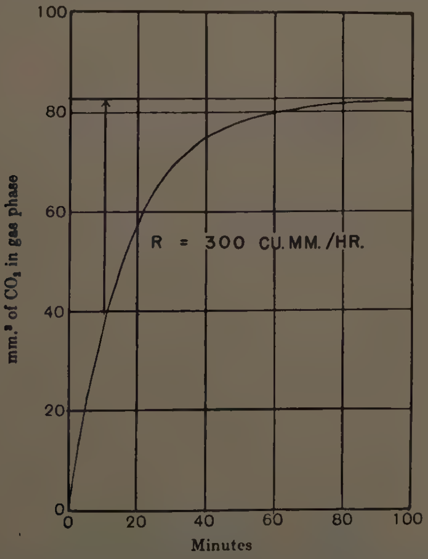
too long a time is required for the building up of the steady-state CO_2 press
correspondingly, the steady-state pressure of CO_2 is relatively quite high
consequence. Thus in FIGURE 9, the 84 cu. mm. CO_2 in the gas phase co
sponds to about 30 mm. Brodie pressure of CO_2 at the steady-state, or ab
about 0.3 per cent-atm., as compared to 0.1 per cent-atm. or less mentio
earlier in connection with a vessel of the type shown in FIGURE 1, with
types of vessels here compared without use of the filter paper device.
comparable respiration rates per volume of vessel, the steady-state pressur
CO_2 would be about six times as high in the vessel of FIGURE 7 compare
the half-as-large vessel of FIGURE 1. Vessels of the type shown in FIGURE 7

much used in that quaint isle to the east of Ireland, but more from patri-
than scientific considerations.

any event, none of the well-vessels containing strong alkali as the CO_2
rbent permits establishment of physiological CO_2 steady-state pressures of



IGURE 8. Rate of absorption of CO_2 in Barcroft vessel under different experimental
itions.[11] Curve A: 40 per cent NaOH in central well, temperature 14° C. (curve for 40° C
tly lower); Curve B: 40 per cent KOH in central well, temperature 40° C.; Curves C and
spectively 40 per cent and 7 per cent KOH in central wells, and also rolls of filter paper in
on right-hand (experimental) side. Values below ordinate of zero, in curves C and D,
ed by oxidation of alkaline filter paper by O_2, and would not have been observed with
tman filter papers 40 and 42, placed in both right- and left-hand flasks.



GURE 9. Curve showing accumulation of CO_2 in the gas phase with absorption condi-
corresponding to curve B of FIGURE 8 (q.v.), and with a CO_2 production of 300 cu. mm.
our by the yeast cells in the medium in the main compartment. Length of arrow indi-
value obtained when tap closed off 10 min. after $t = 0$.

about 5 per cent-atm. for measurement of respiration. To overcome this si
tion, Pardee[3] in 1949 introduced the use of diethanolamine in place of st
alkali, but with only partial success, since (1) about 3 per cent-atm. CO_2
the practical maximum steady-state pressure feasible in any type of manom
vessel, and (2) the time required to reach such a steady-state was still at
10 min. and usually more even in vessels such as that in FIGURE 1, altho
this factor was improved upon by use of Dickens-Simer vessels that provi
considerably greater area of CO_2-absorbing diethanolamine exposed to the
phase (FIGURE 10). The studies of Krebs[4,5] that followed soon afterward
to be recommended as a scholarly consideration of the theory and practi
use of various ethanolamine buffers as CO_2 absorbents in manometry,
respect to both advantages and limitations; gentle warning may be given,
ever, that the treatment of "retention" is unnecessarily complicated an
greatly simplified in the treatment of retention given by Warburg and K
pahl.[7] The vessel types shown in FIGURE 6[9] and in FIGURE 11[6] offer a prac
solution to the problem of the not-quite-adequate equilibration times met
in use of ethanolamines (or carbonates), as compared to strong alkalis in
conventional vessel types. The new vessel type in FIGURE 6 is, in my ex
ence, the most generally useful and satisfactory type of manometric
extant; with a second independent side arm, it will accomplish almost all
special functions of the Dickens-Simer and Dixon-Keilin (Summerson) ves
even for use in differential manometry. The equilibration times attained
the elevated central trough are remarkably short, as shown in Abb. 3, p. 4
Warburg and Krippahl.[9] TABLES 2 and 3 illustrate the use of the vessel
FIGURE 11[6] in measuring respiration and photosynthesis with short equili
tion or transition times; TABLE 2 compares diethanolamine with NaOH,
TABLE 3 shows the sharp transition times obtainable with diethanolamin
one proceeds cyclicly from respiration to photosynthesis and back.

It may be gathered that what is desired in manometry is an equilibr
steady-state that is attained within a few minutes or less. Naturally there
limit of time within which a gas may be shaken in or out of the liquid phas
feasible rates of gas and liquid shaking in the vessels, and with the least in
ference due to geometric obstruction (side arms, wells, and troughs). S
idea of such a practical limit is given by FIGURES 12 and 13, carried out,
respect to O_2 absorption in a "rectangular vessel" without the indicated
metric obstructions; the vessel containing actinometric fluid was illuminate
unshaken condition for 3 min.; during this time about 70 cu. mm. O_2 in
liquid were consumed, and then, in the dark, was shaken at varying speed
permit re-establishment of equilibrium O_2 solubility in the liquid from the
phase. It is seen that a limiting half time of 5 to 8 sec. is possible for prac
attainment, corresponding to virtually complete absorption within abou
sec. at the highest rate of shaking employed, namely at 240 horizontal cy
per minute at an amplitude of 2 cm.; between 135 and 120 cycles there
sharp fall-off in half time. The case of O_2 is given prior to that of CO_2 si
in this example with O_2 only physical considerations are involved, all the ch
cal reactions of O_2 having ceased the instant the light was turned off; whe
with CO_2 there is always the possibility that known or unknown chemica
actions may be additionally involved.

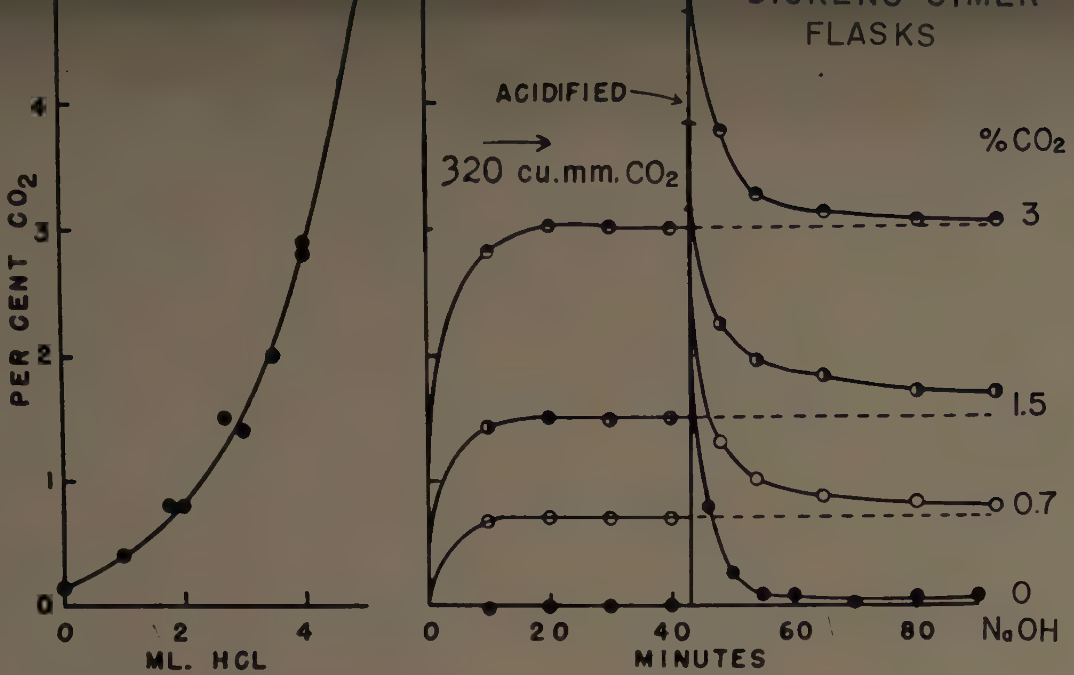
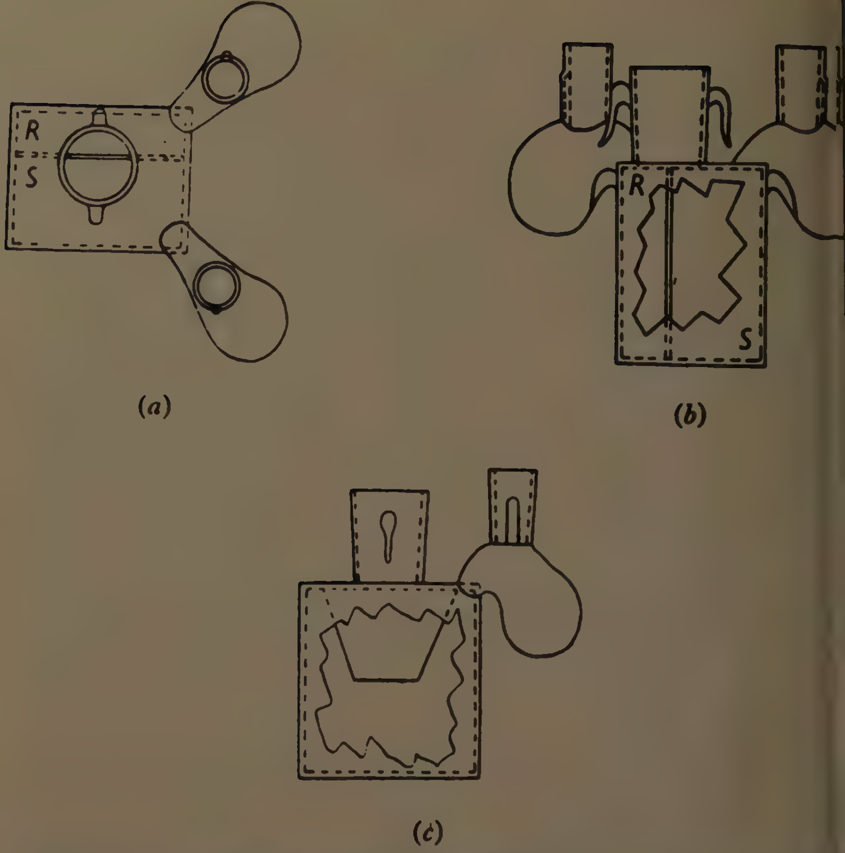


FIGURE 10. (*Left.*) Dependence of CO_2 in gas phase on composition of Pardee solution[3] in central well, as determination by amount of HCl added to diethanolamine-carbonate buffer mixture. (*Right.*) Rate of uptake of 320 cu. mm. CO_2 released at 43 min., after equilibrium CO_2 pressures had been established above various Pardee mixtures or NaOH. Even in the Dickens-Simer manometric flasks employed, the equilibration and times are not quite satisfactory, and vessels of the type shown in FIGURE 6 and 11 are much better.



(a) (b) (c)

FIGURE 11. Two-compartment vessel for measuring with rapid equilibration the pres changes of one gas (for example, CO_2 or O_2) while pressure of the other is kept constant example, O_2 or CO_2 respectively), by respectively oxy-bis(cobaltodiamines) or by dietha amine (or alkalis) placed in the reagent compartment R, with tissue or cells in compartme (*a*) Top view; (*b*) end view; and (*c*) side view. Length (internal dimensions), 35 mm.; wi 28 mm.; height, 38 mm. (or, in another model, 20 mm., with top 18 mm. part eliminat

TABLE 2*

COMPARISON OF RESPIRATION OF *CHLORELLA* AS MEASURED WITH DIETHANOLAMINE NaOH AT CO_2 PRESSURES OF 2.0 AND 0.01 PER CENT-ATM. RESPECTIVELY

Vessel type	FIGURE 11	FIGURE 1
CO_2 absorbent	Diethanolamine†	NaOH‡
Compartment	R	Well
Vessel volume (cc.)	51.0	21.92
k_{O_2}, vessel constant (mm.^{2})	3.85	1.74
Cu. mm. cell/cc. medium (compartment S or main)	40	40
Cu. mm. cells/vessel	280/7 cc.	120/3 cc
Pressure change in dark, 30 min. (mm. Brodie) (fluorescent lighting, 30 min. + 5 min. dark re-equilibration, then):	−29	−28
Pressure change in dark, 30 min. (mm. Brodie)	−29	−27
Total pressure change, 60 min. (mm. Brodie)	−58	−55
Cu. mm. O_2/60 min.	−224	−96
Cu. mm. O_2/60 min./100 cu. mm. cells (= respiration rate as a percentage of cu. mm. O_2/cu. mm. cells/hr.)	−80	−80

* Data adapted from *The Symposium of the Society for Experimental Biology.*[6]
† Diethanolamine: 3 cc. Pardee reagent.
‡ Three tenths cc. 1 *N* NaOH.

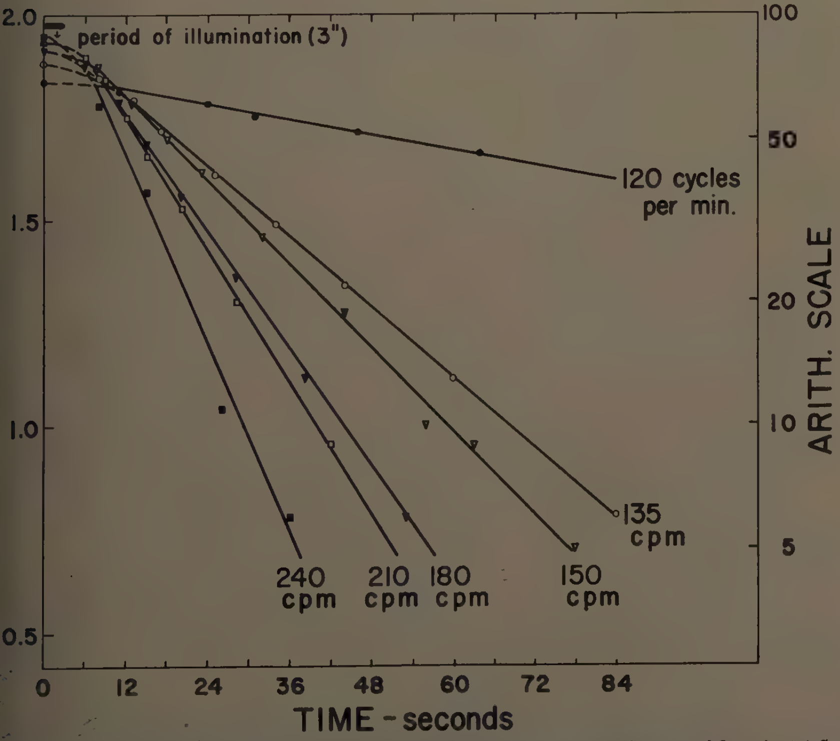
TABLE 3

SPIRATION, PHOTOSYNTHESIS, AND QUANTUM REQUIREMENT OF O_2 PRODUCTION BY *CHLORELLA* AT A CONSTANT CO_2 MAINTAINED BY PARDEE DIETHANOLAMINE REAGENT*

	Pressure change (mm.)	Light action		Quanta absorbed /O_2 produced
		(mm.)	(cu. mm.)	
in. dark	−12			
in. 160 cu. mm. measured ht, 546 mμ	−14	10/20′	38.5/20′	4.2
in. dark	−12			
in. 480 cu. mm. measured ht, 546 mμ	−42.5	28/60′	108/60′	4.4
in. dark	−11.5			
att lamp illumination at 12 hes, 30 min. adaptation, then:				
in. white light	+0.5			
in. white light + 80 cu. mm. asured light, 546 mμ	+5.0	5/10′	19.2/10′	4.2
in. white light	−0.5			

he rate of respiration: (per cent cu. mm. O_2/cu. mm. cells/hr.) — 97.

This is a continuation of the experiment with diethanolamine in TABLE 2 (q. v.) some s later.



GURE 12. Effect of shaking rate on O_2 absorption rate in "rectangular vessel 2 × 4 × 1.5 (k_{O_2}[see legend to FIGURE 1] = 0.70), containing 7 cc. actinometric fluid (7 cc. pyridine ining 300 mg. thiourea, 6 mg. piperidine, 3 mg. ethylchlorophyllide); gas phase, air; erature, 20° C.

Although the half times for CO_2 absorption can be made to approach th of O_2 absorption in aqueous media when comparably optimal conditions set up, we are more interested here in a comparison of the relative rates sho by different CO_2-absorbing media (strong alkali and the various CO_2 buffe To make this study, vessels with troughs (FIGURE 6) were employed, and 1 2 cc. of the CO_2-absorbing media were placed on the floor of the vessel (m compartment); in the trough was placed an appropriate amount of NaH

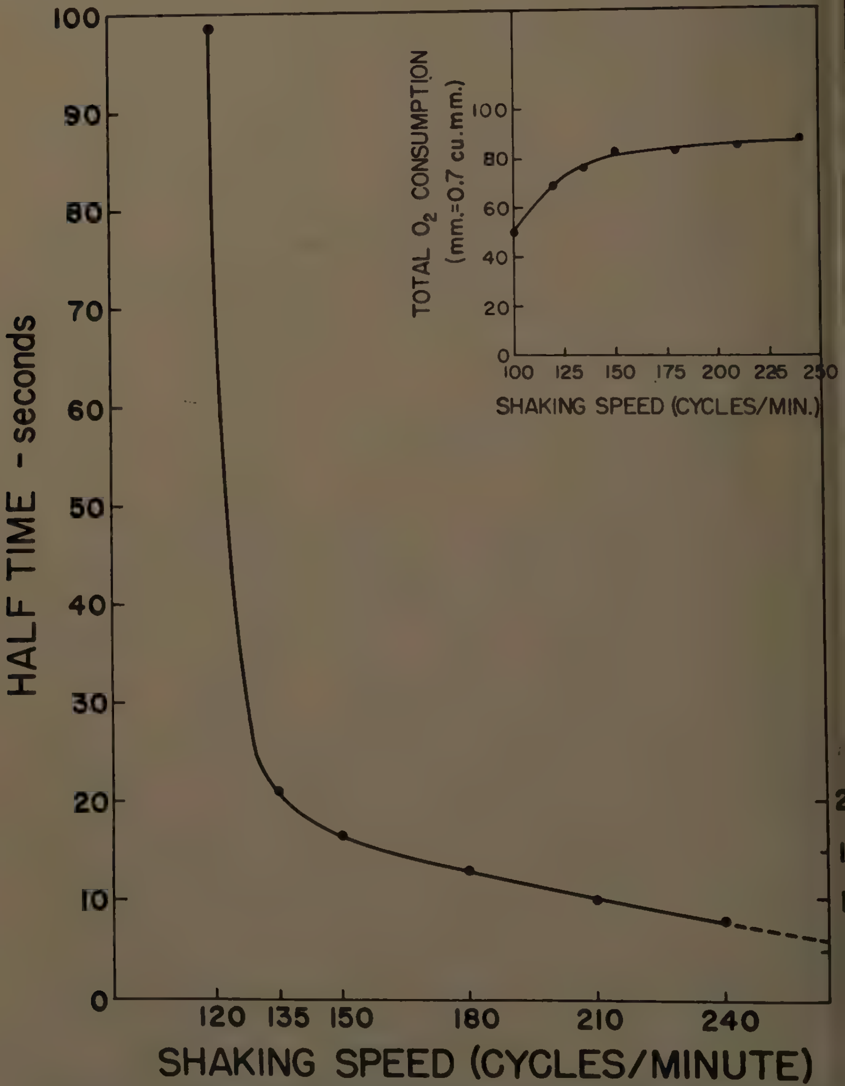


FIGURE 13. Manometric equilibration half times at various shaking rates compared the total oxygen consumed as a function of shaking speed. Replot of data in FIGURE 12

uivalent to 100 to 300 cu. mm. CO_2), and in the side arm was excess 1 *N*
l that was tipped into the trough after manometer-and-vessel equilibration
water bath thermostated at any desired temperature (such as 5, 20, 38, 60,
0° C.). Ordinarily more than 98 per cent of the CO_2 from the $NaHCO_3$
liberated into the gas phase within 1 min. after HCl-tipping and, from then
the first-order (logarithmic) rate of absorption was measured and plotted
semilog versus time coordinates, from which the half times could be read off
dily. The half times found experimentally at 38° C. under such an arrange-
nt varied from 40 sec. to about 3 min. They were least with strong alkalis
example, 1 to 4 *N* KOH or NaOH); somewhat greater with monoethanol-
ne (25 vol. per cent in water) and carbonate buffer (such as 2 *N*, 20 per
t bicarbonate, potassium salt) containing carbonic anhydrase; and greatest
h the diethanolamine, Tris buffer, and the carbonate buffer without carbonic
ydrase. Many variations of this condition were studied without notably
nging the relative order of half-times for the different CO_2 absorbents.

he amine and carbonate buffers were also studied by a modification of the
ple method of Philpot and Philpot,[15] with special reference to the influence
arbonic anhydrase, and all buffers tested were found to respond markedly
this enzyme. Care was taken always to have enough $KHCO_3$ present to
l the initial *p*H below 11, above which *p*H carbonic anhydrase is in due
rse destroyed. In view of the fact that all buffers tested, whether ordinarily
ver found in living matter, responded to carbonic anhydrase, it would ap-
r that the chief specific action of carbonic anhydrase is to catalyze the non-
c equilibrium between H_2O and CO_2 and H_2CO_3, rather than any of the in-
nerable ionic possibilities (except that between CO_2 and OH^-). The method
Philpot and Philpot[15] was developed by them to measure relative carbonic
ydrase in different materials, and was carried out at a given temperature
C.) and with a given carbonate-bicarbonate mixture (0.3 *M* Na_2CO_3–0.21
$NaHCO_3$) in order to obtain results expressible in arbitrarily defined activity
ts. In the present work, on the other hand, the method was adapted to
asure relative CO_2-absorption rates by various buffers, and was carried out
any number of arbitrary carbonic anhydrase concentrations and tempera-
es, although usually at room temperature. Some 10 cc. of a very weak
arbonate solution (about 0.003 *M*) containing any convenient amounts of
bonic anhydrase and of an indicator (such as phenolphthalein, cresol red,
nol red, or brom thymol blue), were added to an oversized test tube, and
k CO_2 gas was passed through vigorously via a pipette in order to attain
maintain saturation of the solution at 100 per cent-atm. CO_2 (or any other
ired percentage) during the experimental run. After a minute or two of
sing, a given concentration or amount of buffer to be tested was added by
watch, and the time required for titration back to the acid side of the in-
ator was measured and compared to the time required in the absence of
bonic anhydrase. As indicated earlier, carbonic anhydrase, depending upon
concentration, can shorten the required titration time (1 to 3 min. in the
ence of carbonic anhydrase) by orders of magnitude. The differences be-
en various buffers were generally less by this method than by the mano-
ric method, presumably because the catalytic reaction involved takes place

in a homogeneous liquid, and involves no heterogeneous gas-liquid reacti
the rate limiting stage, as is possible in the manometric experiments alr
described; the homogeneous reaction is more exactly confined to the cata
limitation phase (presumably chiefly nonionic) without complication by h
ogeneous diffusion aspects. For this reason, in addition to the greater
plicity of apparatus, such a modified Philpot-and-Philpot method is prob
more suitable and more direct for use in comparing the effect of carboni
hydrase on various buffers that might be studied in connection with la
scale CO_2 scrubbing, as in those employed on submarines or in industrial p
esses generally. Indeed, in so far as possible application of carbonic anhyd
to such scrubbers is concerned, the scrubbers themselves presumably woul
the most suitable forms of apparatus wherein to conduct the tests, rather
in test tubes, especially where mixing of gas and liquid is carried out differe
than in the simple test-tube test. Thus, whereas in the test-tube method
gas is passed through otherwise stationary liquid, in the submarine abso
(FIGURES 3 and 4) the scrubbing is concurrent (air-blown and entrained in
same direction as the absorbent). In contrast, in many industrial proce
the scrubbing is countercurrent (gas and liquid passed in opposite directi
instead of bubbles of gas in the test-tube method, thin moving turbulent f
in packed towers are often involved.

Case 2. Equilibrium steady states. According to Willard Gibbs and G
Lewis, all thermodynamic equilibria are really steady states, so no apolog
needed for the title of this section if one is willing to accept these authoriti
authoritative. The immediate manometric problem is to define what parti
bicarbonated CO_2 buffers in solution will produce desired equilibrium
pressures in the gas phase in the vessel, which gas pressures will, in turn, de
mine the CO_2 content of the (organism-containing) medium also in the ve
but out of physical contact with the buffer. These processes, preferably, sh
be accomplished so as to involve no practical aspect of time, or the grea
possible minimization of it. Obviously if there is any difficulty on this la
score, carbonic anhydrase, from what has been reported here, could be of g
assistance in innumerably possible situations involving CO_2 gas and its hos
subsidiary reactions with various buffers.

Most plant and animal cells are best supplied, in nature or in laboratory
periments, with 5 per cent-atm. CO_2 in the gas phase, although, commo
they function equally well with somewhat lower pressures. As Pardee[3]
Krebs[4,5] have shown, diethanolamine, almost uniquely among a wide variet
amines tested by them, will satisfactorily supply up to 3 per cent-atm.
at most common physiological temperatures, but at 5 per cent-atm. CO_2
retention requirement and certain other aspects are not satisfactorily cov
even by diethanolamine. On the other hand, as earlier indicated from
work of Warburg and Krippahl[7] and myself, aqueous carbonate-bicarbo
mixtures, especially in the presence of carbonic anhydrase, can supply rea
not only 5 per cent-atm. CO_2 but also pressures that are orders of magnitud
either side of this value, merely by changing the ratio of carbonate to b
bonate and/or the total molality. For the higher pressures of CO_2, quite
concentrations of buffer are required (1 to 4 M) and, for this reason, the
tassium salts are generally preferred to the sodium salts, since $NaHCO$

ially has very considerably less solubility than $KHCO_3$; for low pressures
O_2 the distinction between potassium and sodium salts is not very material.
ccording to my measurements, carried out by methods to be described in
next section, 5 per cent-atm. CO_2 in the gas phase will, at body temperature
C.), be provided by (be in equilibrium with) a mixture of close to 80 parts
M $KHCO_3$ and 20 parts of 3 M K_2CO_3 ; the same mixture at 20° C. will
vide about 2.5 per cent-atm. The corresponding values for a 70:30 mix-
e are 3 per cent-atm. at 38° C. and 1.5 per cent-atm. at 20° C. The temper-
re coefficient here is thus about 2 per 18° C.; other experiments carried out
r the range 1° to 90° C. indicate that this temperature coefficient between
and 38° C. is not a marked function of temperature, although possibly it is
ttle larger at lower temperatures. Warburg and Krippahl[7] have recently
lished a provisional table of equilibrium CO_2 pressures and retention values
various carbonate-bicarbonate ratios between 5/95 and 85/15 and for molari-
between 0.1 and 3.0, for the temperature of 20° C., but they will shortly
ivate communication) publish a more accurate set of values. Their pro-
onal values are about 50 per cent higher than those determined by me, and
cause of this discrepancy has now been jointly determined to our mutual
sfaction. Apart from any consideration of absolute equilibrium values,
published table of Warburg and Krippahl provides an exceedingly useful
r-all view of the approximate relative effects of buffer concentration and
bonate-bicarbonate ratio; the true equilibrium values are about two thirds of
se reported in the table, at least for the higher CO_2 pressures.

ase 2 continued: Measurement of equilibrium CO_2 pressures at steady state.
ave studied a wide variety of manometric methods for determining equi-
ium CO_2 pressures above carbonate-bicarbonate mixtures and amine buffers.
o of the most suitable procedures, depending upon type of vessel employed,
be outlined. At the outset it may be stated that it is safer to employ meth-
that involve no *flowing* systems, since these systems always require further
of that the gas coming off or out of a buffer mixture has had time fully to
ilibrate and, in general, this will seldom be the case; any methods that in-
ve time as a factor are to be avoided for the most exact work. Moreover,
solid buffer salts and liquid medium must be premixed to avoid large cor-
ions for changes in water-vapor tension, dilatometric effects, and changes
olume due to mixing two or more materials, heterogeneous or homogeneous.
most accurate work, it is necessary to analyze carefully even the "analytical
de" reagents employed; most bicarbonate salts will slowly accumulate car-
ate due to simultaneous formation of CO_2 , so it is best to pass CO_2 through
prepared stock bicarbonate solution before analyzing it for exact bicarbonate
tent (freed of carbonate by passing the CO_2).

he best general method, in my experience, is to place such a certified buffer
ture (with or without carbonic anhydrase) in some part of a vessel that may
ept closed off from the main gas phase until after the manometer and vessel
e been fully temperature-equilibrated and water-vapor-equilibrated in the
rmostat bath. The main compartment of the vessel should contain a small
ount of water to facilitate rapid establishment of the equilibrium water-
or pressure, and the gas space should be filled with CO_2-free gas, or air where
3 per cent-atm. is immaterial. Dixon-Keilin or Summerson vessels (FIGURE

14) are suitable vessels, since their bottom plugs can be filled with buffer turned to connect with the gas phase only *after* temperature and water v equilibration; similar control vessels, with, for example, water instead of bu should be run at the same time, in addition to the standard thermobarom (T.B.). Upon turning the plug, the maximum pressure developed repre the equilibrium CO_2 pressure (each 100 mm. Brodie pressure developed gardless of vessel size!] representing 1 per cent-atm. CO_2 in the gas ph

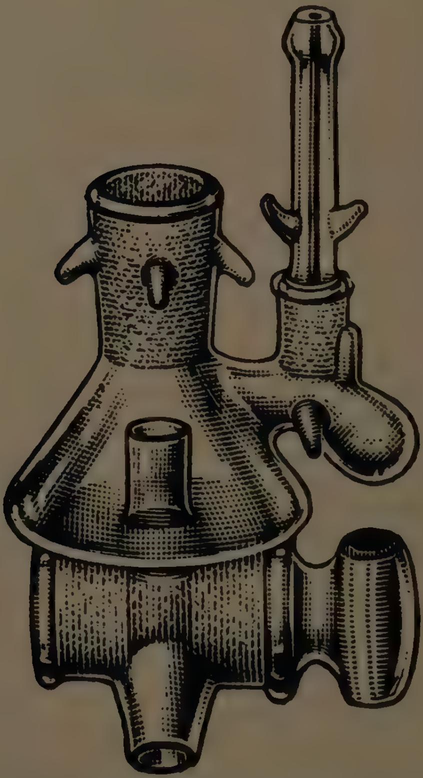


FIGURE 14. Summerson (or Dixon-Keilin) type of manometric vessel, especially use differential manometry, with lower-stopcock plug that can be filled with CO_2 absorbent t not connected with the gas phase of the vessel until after temperature and water-vapor libration is completed.

subject only to quite minor correction for any pressure that may devel the plug-with-water control; in my experience such correction has almost al been zero within experimental error. It is further possible, if one has both experimental vessel and its control vessel attached to a single Summe manometer, *differentially*, to take the manometer out of the water bath equilibrium has been established and measured for the temperature of that and place the manometer in another water bath at another temperature obtain the new equilibrium CO_2 pressure for the second temperature, and in way to other temperatures. Final return to the original temperature sh

l the original equilibrium CO_2 pressure. One can thus study a whole series
emperatures with one and the same differential manometer. The mighty
nmerson manometer, used *differentially*, has the further advantage that, by
ber adjustment of both inner arm settings, one can handle ranges up to 10
cent-atm. CO_2 (readings of 1000 mm. Brodie).

he second type of procedure is based not on the evolution of CO_2, as above,
on its absorption after equilibrium has been established. Instead of only
er being placed in the main compartment, one adds several tenths of 1 cc.
eutral or slightly acid solution of $KMnO_4$ (2.5 to 5 mg.); in the side arm one
es several tenths of 1 cc. of neutral or slightly acid solution of $K_4Fe(CN)_6$
to 40 mg.); the same procedure is followed in the control. After the equi-
ium described in the foregoing paragraph has been attained, the plug is
ed off again and the ferrocyanide is then mixed into the permanganate.
decrease in pressure observed in due course (corrected for any similar but
h smaller pressure change in the corresponding control) should equal the
inal positive pressure developed: both methods should agree. The use of
ocyanide-permanganate mixture for forming alkali to absorb CO_2 is de-
bed in detail by Warburg and Krippahl.[9] It may be added that experience
shown that as long as the plug has 0.2 cc. or more of buffer, the equilibrium
lts obtained are unaffected by volume of buffer. This should be the case
e, ordinarily, only a relatively small percentage of the potential CO_2 in the
er goes off into the gas phase, leaving the buffer mixture essentially un-
nged in composition.

further variant of the second-cited method can be performed with any
nary manometric vessel equipped with two side arms, provided that the
er are large enough to hold adequate amounts of reagents. Into such a
sel is put about 1 cc. of CO_2 buffer in the main compartment, in one side
about 1 cc. of nearly saturated $K_4Fe(CN)_6$ and, in the other side arm, an
ivalent amount of $KMnO_4$ solution (1 mole $KMnO_4$ per 3 moles $K_4Fe(CN)_6$,
ield 4 moles KOH equivalent). After complete temperature equilibration
he water bath at any desired temperature, the ferrocyanide and perman-
ate are tipped in with the buffer mixture and the final equilibrium pressure
ead in due course. The decrease in pressure (corrected for a control ex-
ment in which the carbonate-bicarbonate buffer is replaced by carbonate
tion of the same total molality) is a measure of the equilibrium CO_2 pres-
that existed before tipping. The total alkali formed from the ferrocyanide
permanganate must, of course, be greater than the total CO_2 that had
n in the gas phase plus the (still greater) bicarbonate in the buffer, but this
ot difficult to arrange. The final *p*H of the total mixture should be above
o insure complete absorption and retention of all CO_2 from the gas phase
ention greater than 100). With highly concentrated carbonate-bicarbonate
er solutions yielding high equilibrium pressures of CO_2 before tipping, the
sure change in the corresponding carbonate control will not be strictly
igible, especially at higher temperatures, will usually be of positive sign
oosite to that of the readings with buffer), and must be added algebraically
he latter (a water rather than carbonate control yields a smaller pressure
nge, one that is negative, like the buffer change). The pressure change in
carbonate control, representing a volume change due to mixing of two con-

centrated reagent solutions, is very much smaller than what would be obta
through the use of solid reagents mixed with water, since, being alread
solution, the dilatometric, water vapor, and mixing effects are thereby alre
greatly reduced. One may check not only the final *p*H, as would be expec
but also make a direct determination of retention on the final total mixture
confirm the existence of some retention value between 100 and infinity.
retention of 100 means that the CO_2 pressure above 1 cc. of solution contai
100 cu. mm. CO_2 will be 1 mm., the units of retention being cu. mm. CO
tained/mm. pressure/cc. solution.[7] For an excellent brief description of
tention, especially as applied to the problems under consideration in this pa
reference may be made again to the article of Warburg and Krippahl[7] (pp.
367). TABLE 4 is a protocol of a typical experiment made with the two-s
arm vessel variant method.

TABLE 4

DETERMINATION OF EQUILIBRIUM CO_2 PRESSURE IN GAS PHASE ABOVE TWO BICARBONATE-CARBONATE MIXTURES AT TWO TEMPERATURES

3 *M* $KHCO_3/K_2CO_3$	Temp. (°C.)	80/20	70/30	0/100 (carbonate control)	0/0 (wate
h, pressure change after tipping ferrocyanide and permanganate (mm. Brodie)	20	−252	−136	+15	−6
	38	−498	−275	+37	
Per cent-atm. CO_2 (after correction for carbonate control)	20	+2.67	+1.51		
	38	+5.35	+3.12		

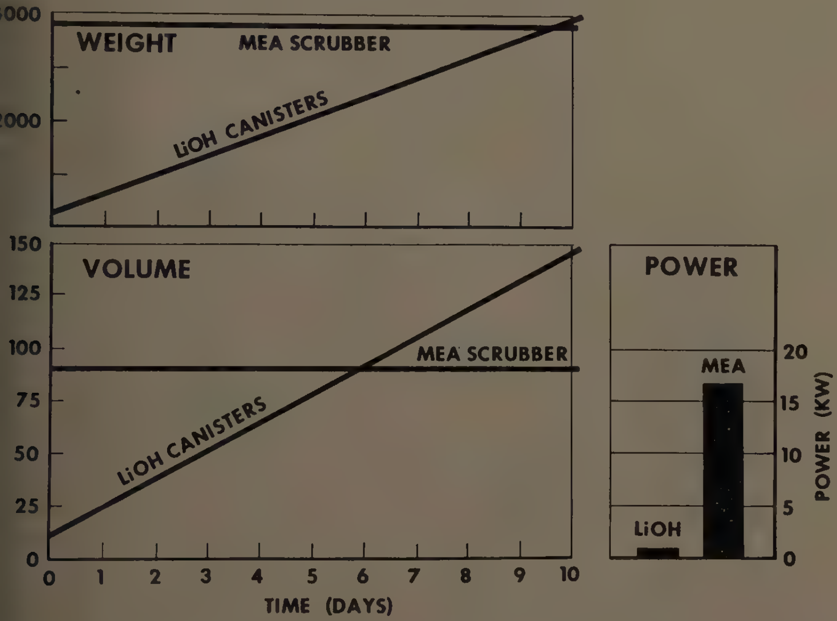
The final *p*H: 10.2 to 10.7 for buffers (80/20 and 70/30), >12 for carbonate co
(0/100), and water (0/0).

Retention in all: 100 to infinity (cu. mm. CO_2 retained/mm. pressure CO_2/cc. bu
mixture).

Submarine and Industrial CO_2 Scrubbers

As indicated in FIGURES 1, 6, 7, and 14, the CO_2-absorbing well or troug
not a very large fraction of the volume of the manometric vessel. Simila
as indicated in FIGURES 2, 3, 4, and 5, the CO_2-absorbing scrubber or cani
is an even still smaller fraction of the total volume of the atomic (nucl
powered) submarine. As indicated in FIGURE 15, the nonregenerative Li
canister enjoys a temporary and short-lived advantage of volume and wei
over the regenerative monoethanolamine scrubber, but how much FIGURE
might have to be redrawn if carbonic anhydrase could be made useful is
interesting question that will be given some brief attention here. In the c
rent standard book on gas purification by Kohl and Riesenfeld,[16] carbo
anhydrase is not listed in the index, nor could any reference to it be found
the text, which in various places clearly suffers from a lack of consideratio
the possible action of this enzyme. It is commonly stated that monoetha
amine is a better absorber of CO_2 ratewise than is K_2CO_3, but this statem
holds true with certainty, to my knowledge, only in the absence of carbo
anhydrase, not necessarily in its presence; this last point remains for determ

with respect to any of a variety of circumstances that might be of interest. is no secret that submarines of the German Navy in World War II prob- suffered more, directly *and* indirectly, from inadequate elimination of CO_2 n the air presented to the crews than from adversary arms or attrition. relatively successful adaptation of the use of monoethanolamine CO_2 ubbers for nuclear-powered submarines, fortunately just prior to, rather n somewhat after, these ships became a reality, has led to a certain amount esting upon laurels that is certainly justified if one looks backward, but haps not if one looks forward. For monoethanolamine, that currently best



IGURE 15. Comparison of system characteristics of monoethanolamine scrubber and H canister for CO_2 removal (based on 100 men).[2]

wer to a submarine engineer's prayer, still suffers from a number of ac- wledged drawbacks:[2] it is corrosive for steel; it has a relatively high vapor sure; it presents an atmospheric toxicity threat; it produces ammonia; it isceptible to oxidation and thermal decomposition, with a consequent re- ement for not-infrequent periodic replacement; it displays a possible irement for added chelating agents to reduce amine degradation; and it lves messiness in handling.

he shortcomings that are *not* cited in this list may be not only the most im- ant, but also those about which most could be done: they include the fact it is still true that a much higher rate of absorption of CO_2 by the mono- nolamine mixture would be desirable; its rate is greatly reduced because er the conditions of its operation it is already about 80 per cent neutralized

by CO_2. Hence only about one fifth of the ethanolamine is actually avail
for kinetic reaction with CO_2. It remains to be ascertained whether, un
operating scrubber conditions, carbonic anhydrase would cause the same o
of manifold increase in rate of CO_2 pick-up by monoethanolamine that I
served by the modified Philpot-and-Philpot method described earlier in
paper; at the moment of writing there exists no basis of contraindication.
short, such experimentation to utilize carbonic anhydrase for some form
scrubber action improvement is the simplest and the first that should be tr
involving, as it does, nothing more complicated than adding exceedingly sm
quantities of enzyme to the absorber (FIGURE 16), without any further engin
ing changes in design. Any carbonic anhydrase transported from the abso

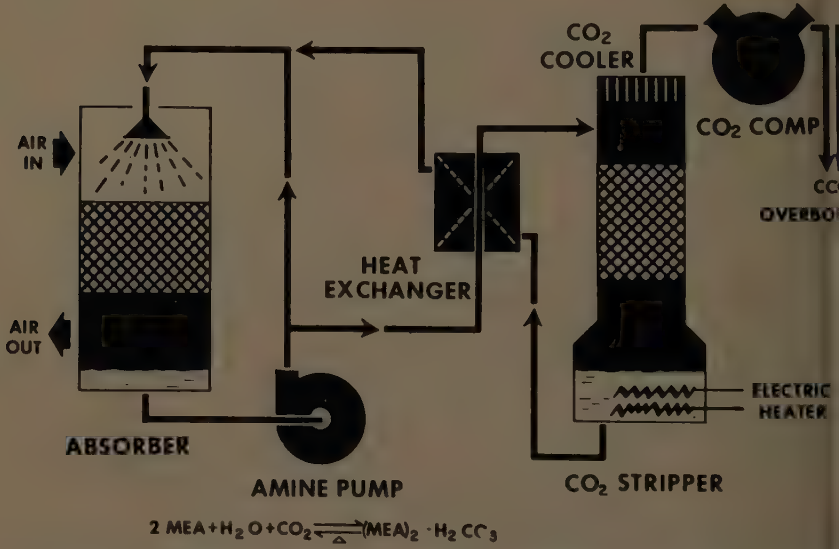


FIGURE 16. Schema of CO_2-removal plant of monoethanolamine type employed
nuclear-powered submarine.[2]

(at about 90° F.) to the stripper (at about 270 to 280° F. as now operat
would necessarily be destroyed at the higher temperature and have to be
placed in the absorber from time to time; "from time to time" because
roughly 36 gallons of monoethanolamine mixture in the absorber is sent to
stripper at the relatively slow rate of only 1.25 gal./min. The average
placement time would thus be about one half hour if one started initially
the minimum amount of enzyme to provide the maximum desired rate sti
lation; but if one started with, for example, 10 times the minimum amount,
replacement periods might be extended to several hours providing there
no extensive inactivation of the enzyme under operating conditions, a fa
about which there is at present no knowledge. Carbonic anhydrase is on
the most stable enzymes, fortunately, with remarkably wide limits of to
ance with respect, particularly, to *p*H and temperature. Carbonic anhyd
is also highly active per unit weight of enzyme; according to one of its

overers,[17] one part in 10,000,000 (by weight) produces marked acceleration
O_2 exchange. In a 36-gal. mixture this would correspond to about 15 mg.
ified enzyme to be added per one half hour if, as is roughly true in my ex-
ence, the amounts of enzyme required with monoethanolamine are about
same as with the carbonate-bicarbonate systems. In practice the amount
uired could well be severalfold different on *either* side of the 15 mg. value
several reasons that need not be discussed here, but presumably the order
agnitude is correct. Fortunately there are several industrial supply firms, in
United States and elsewhere, that make highly purified carbonic anhydrase
arations commercially at prices governed to a large extent by demand.
or sources of supply, red blood cells of various animal species are about the
est, and these are available in essentially unlimited quantity. Red blood
s, by themselves, are of the order of 1/500 to 1/1000 as active per unit of
ght as is the purified enzyme, depending upon the animal species; human
d, for example, is one half as active as ox blood, and could conceivably in
emergency be used in place of purified enzyme in a CO_2 scrubber were it
for eventual "crudding" of the buffer mixture and apparatus. For short
s it might suffice, especially if the red cells were first separated from the
of the blood. If carbonic anhydrase actually should accelerate the CO_2
orption rate markedly in a monoethanolamine scrubber, it goes without
ing that engineering modifications and simplifications would probably
ow to take the greatest advantage of the acceleration. Even if this problem
e solved, one would still be faced, of course, with most of the "drawbacks"
d above.

Vith the last observation in mind, together with the many advantages noted
anometry of carbonate-bicarbonate buffer systems over ethanolamine sys-
s, I here propose detailed investigation of the carbonate-bicarbonate buffer
ems to replace monoethanolamine in submarine (or industrial) CO_2 scrub-
s, *using carbonic anhydrase as catalytic adjunct*. First, one might simply
ace the monoethanolamine mixture by a predetermined suitable mixture
arbonate and bicarbonate, keeping the rest of the plant the same, or per-
s altering the scrubber and stripper temperatures; the latter, for instance,
rding to equilibrium measurements I have made, might perhaps be reduced
n about 270° F. to somewhat below 212° F., even though the scrubber
peratures were kept unchanged at about 90° F.

much more ambitious and long-range proposal, one that I prefer as po-
ially capable of leading to a marked change and simplification of engineer-
design and methodology, would be to study use of carbonate-bicarbonate
bbing and stripping of CO_2 *with carbonic anhydrase as catalytic adjunct*,
operation of the absorber at, let us say, 5° C. and the stripper at 60° C.,
peratures markedly different from those now in use, most notably with
ect to the lower temperature. The temperature of 5° C. (instead of even
lower) would appear to be determined primarily by engineering considera-
s, since the enzyme itself would work well below this temperature; the
er temperature of 60° C. is set by the enzyme-stability limitation (unless
might use still higher temperatures without participation of the enzyme,
n one of the earlier proposals above). According to equilibrium measure-
ts I made, a mixture of 40 parts of 2 N $KHCO_3$ and 60 parts of 2 N K_2CO_3

has an equilibrium pressure of about 0.1 per cent-atm. CO_2 at 5° C. and
per cent-atm. CO_2 at 58° C.; other mixture ratios and total normalities c
be found to give approximately the same results also. At any rate,
absolute pressures are very close to what is desired if the present monoethan
mine system is to be matched as to pressures going in and out of the absorb
FIGURE 16, namely, respectively about 1.0 to 1.2 per cent-atm. CO_2 at th
take and 0.1 to 0.2 per cent-atm. CO_2 at the outlet. Another important as
that must be matched is the ratio of water-vapor pressure to CO_2 pressure
above the stripping mixture. This is important because the lower the
the less will be the power requirement for separating the water from the
beyond the stripper. The ratio in the currently employed apparatus
monoethanolamine at 270° F. (FIGURE 16) is of the order of 15 to 20; accor
to my measurements the ratio with the above-mentioned carbonate-bicarbo
mixture is probably somewhat less, that is, probably a little more favorabl

From equilibrium considerations, then, the 5° C. to 60° C. carbonat
carbonate-carbonic anhydrase system seems potentially feasible. There
remain, of course, rate considerations to be resolved experimentally in ap
priate scrubber apparatus. To match the current requirements and op
tion, the proposed system at 5° C. would need to take in 250 cu. ft./
of air and absorb some 2.5 cm. ft. of CO_2 NTP/min., or about 70 l. CO_2 N
min., in a nuclear-powered submarine of the *Nautilus* prototype with about
men aboard. Expressed otherwise, the 5° C. buffer system would nee
absorb about one half its volume of CO_2 per minute to match the cur
monoethanolamine performance at 90° F., and would need to do so at
pressures varying between 1.2 and about 0.2 per cent-atm. (intake and ou
pressures). To judge from my comparisons of monoethanolamine and
bonate-bicarbonate-carbonic anhydrase systems by the modified Philpot-
Philpot method, this should be feasible, but of course final test must aw
scrubber of the type shown in FIGURES 3 and 4. To compensate for
known higher absorption rate with monoethanolamine as compared to
bonate-bicarbonate *without* carbonic anhydrase, there will be, of course,
great acceleration of the carbonate-bicarbonate system by carbonic anhyd
and the advantage of the considerably greater solubility (over twofold
CO_2 at 5° C. than at 90° F., which could, conceivably, give even greater
for the carbonate-bicarbonate system with carbonic anhydrase as compare
the rate for monoethanolamine without carbonic anhydrase at the respecti
different temperatures under consideration. On the other hand, the m
ethanolamine system has the advantage of higher operating temperatur
favor its rate.

The three chief proposals may be summarized, in the order of immedia
long-range: (1) add carbonic anhydrase to the current monoethanolamine
sorber system (FIGURES 3 and 4); (2) replace monoethanolamine by an
propriate carbonate-bicarbonate-carbonic anhydrase mixture, with pos
variant of temperature changes, especially the stripper temperature; and
use a carbonate-bicarbonate-carbonic anhydrase system between about 5°
60° C. instead of about 90° and 270° F.

n conclusion, and to return to the opening theme of this paper, it is inter-
ng to note that equations **1**, **2**, and **3** in essence describe equally well the
dy-state pressures of CO_2 in the gas phases of both the manometric vessel
URE 1) and the atomic submarine (FIGURE 2), in spite of their 200,000,000-
l difference in size; that both these types of (submersible!) vessels involve the
e types of, and problems regarding, CO_2 absorbents; and that carbonic an-
rase has much potential promise for both with respect to the buffer type
O_2 absorbers. Detailed applications to industrial scrubbers have been left
ely implicit, but perhaps potential promise and extended application is
n greatest here.

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poration, Groton, Conn. FIGURES 2, 3, 4, 5, 15, and 16 were kindly sup-
d by the Bureau of Ships through the courtesy of William McConnaughey.
a pleasure to acknowledge the earlier collaboration of Hans Lineweaver on
erial in the section on *Absorption rate steady-states*.

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LOOD pH AND $p\text{CO}_2$ HOMEOSTASIS IN CHRONIC RESPIRATORY ACIDOSIS RELATED TO THE USE OF AMINE AND OTHER BUFFERS

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ysiology Branch, Medical Research Laboratory, United States Naval Submarine Base, New London, Groton, Conn.

This report deals with the use of amine buffers in the CO_2 scrubber of sub- arines and the problems of chronic CO_2 toxicity as they relate to treatment th buffers. I propose to discuss the CO_2 control of the submarine atmos- ere very briefly, since I can only provide some general information on this bject based on the work of engineers at the Naval Research Laboratory, ashington, D.C. I shall present data on the homeostasis of pH and $p\text{CO}_2$ tained during the prolonged exposure of 23 subjects to 1.5 per cent CO_2 that dicate the effects of elevated $p\text{CO}_2$ independent of pH changes. Only the tter are subject to treatment with amine buffers. Moreover I shall mention rlier attempts to use alkalinizing agents, such as potassium salt, to increase e tolerance to hypercapnia.

Atmospheric Control with CO_2 Scrubbers Using Amine Buffers

Chronic CO_2 toxicity is known to have been a cardinal problem of submarine edicine for many years, simply because no technical advances were made to velop efficient self-regulating CO_2 removal equipment. With the advent of iclear-powered submarines capable of greatly extended submergence times, was necessary to find a solution. The introduction of a CO_2 scrubber using onoethanolamine represented the first satisfactory accomplishment to remove $)_2$ with self-regenerating absorbent. Monoethanolamine (MEA) absorbs $)_2$ at room temperature and releases it at higher temperature. A simplified agram of the amine-type CO_2 removal equipment is presented in FIGURE 1. consists of an absorber column, a CO_2 stripper, an amine pump, and a heat changer. The submarine air is pumped through a cool MEA solution in the sorber column and CO_2 is chemically absorbed. The MEA solution is cycled through the absorber column. Part of the MEA solution is piped to e CO_2 stripper, where it is heated under pressure to release the CO_2. The ter is further compressed and discharged overboard. The hot MEA is oled and used again in the absorber. The CO_2 scrubbers presently in opera- on have a capacity to remove 10 lb. of CO_2/hour while operating at 170 c.f.m.

FIGURE 2 shows a comparison of a conventional lithium hydroxide (LiOH) rubber used on battery-powered submarines with an MEA system in regard weight, volume, and power requirements. It is obvious that the LiOH stem has an advantage for short periods up to 10 days. For the longer riods, the MEA scrubber is of course the better choice.

According to Goodridge,[2] it is believed that mono- and diethanolamines ict with CO_2 by donating an electron of a hydrogen molecule from the amine kage and formation of carbonates. Since the molecular weight of mono- ianolamine is much smaller than that of diethanolamine and triethanolamine

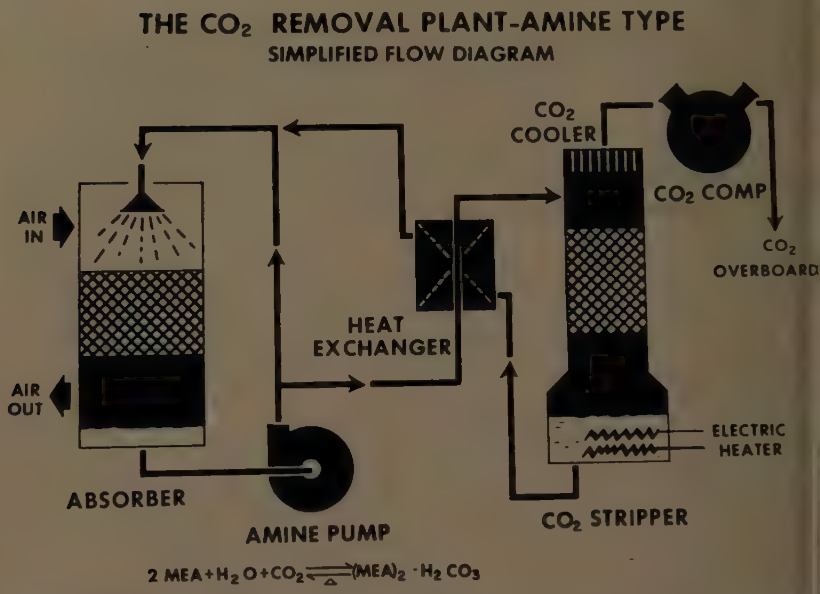

FIGURE 1. Diagram of an amine-type CO_2 scrubber. (Reproduced by permission The Macmillan Co., New York, N.Y.[1])

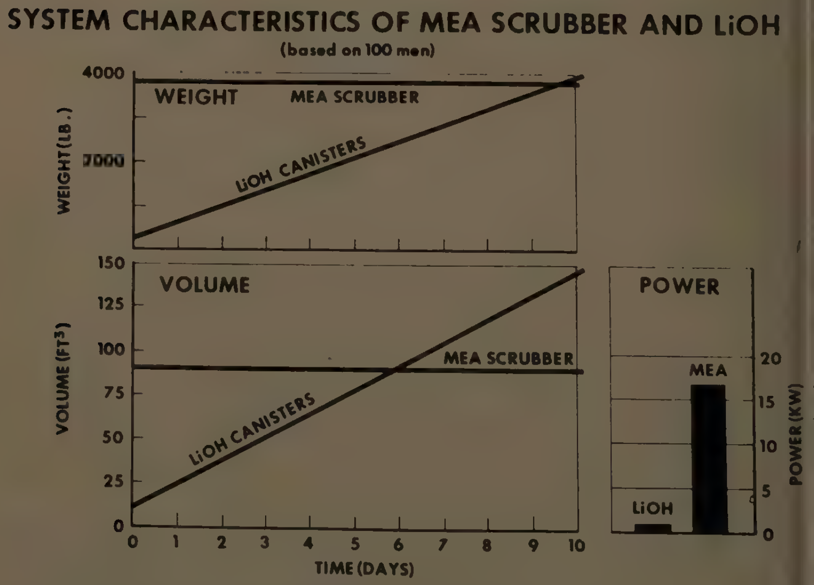

FIGURE 2. Weight, volume, and power requirements of MEA and LiOH CO_2 scrubl calculated for 100 men. (Reproduced by permission of The Macmillan Co., New York, N.

l only 1 mol. of amine is utilized per molecule of CO_2 reacted, the relative orption capacity of CO_2 per pound is much greater for MEA (1.0:0.58:0.41). ;A leaves something to be desired as a regenerative carbon dioxide absorbent. ıas a lack of resistance to oxidation that in time leads to a degradation of MEA solution requiring replacement. Furthermore, MEA has a certain icity. The current maximal allowable concentration (MAC) established the Bureau of Medicine and Surgery for the United States Navy is 1 ppm.*

Blood pH and $p\mathrm{CO}_2$ Homeostasis During Prolonged Exposure to Increased CO_2 Levels

n World War II, when no adequate CO_2 removal equipment existed in marines to allow prolonged submergences, CO_2 concentrations of 3 to 5 cent had to be tolerated over extended periods. It was during this time t interest was developed in using alkali buffers to make men CO_2 resistant l, eventually, to raise the tolerance to CO_2 in submarine crews. These ly attempts to treat respiratory acidosis with Na and K buffers will be :ussed later. I shall concentrate first on the results of studies that produced dence that the effects of increased $p\mathrm{CO}_2$ independent of pH changes are ıificant. In emphasizing the aspects of $p\mathrm{CO}_2$ homeostasis, which is not uenced by alkali or Tris buffer medication, it is hoped that a useful contribu- ı may be made to define the limitations and indications for the use of Tris fer in respiratory acidosis.

n a large experiment 23 subjects were confined in a submarine and exposed l.5 per cent CO_2 in 21 per cent O_2 over a period of 42 days, with a 9-day trol period prior to and following exposure. The results showed no significant changes in performance[3] or in basic physiological parameters, such as od pressure, pulse rate, weight, and body temperature (FIGURE 3). However, studies of respiration,[4] acid-base balance, and electrolyte exchange[5] ught some unexpected findings. A slight uncompensated respiratory losis existed for a period of 23 days after which the pH returned to normal. ring a 9-day recovery period following a 42-day exposure to 1.5 per cent $_2$ the alveolar $p\mathrm{CO}_2$, blood $p\mathrm{CO}_2$ remained elevated at the level reached ing exposure. The existence of a phase of uncompensated respiratory losis for 23 days followed by a phase of compensated respiratory acidosis n the 24th to the 42nd day is indicated in FIGURE 4 in the changes of pH, olood and urine, and CO_2 excretion in the urine. Moreover a significant ression of the respiratory response to 5 per cent CO_2, previously described art of a respiratory adaptation to CO_2,[6] was found after 35 days of exposure l.5 per cent CO_2 (FIGURE 3). The central importance of the respiratory tern in adaptation to CO_2[7] was demonstrated in a progressive increase in l volume throughout the 42 days of exposure and a decline of respiratory : during the second phase subsequent to a transient increase during the : (uncompensated) phase of respiratory acidosis. This pattern of respira- ı is so strongly established at the end of a 42-day exposure to 1.5 per cent $_2$ that transition to air is not able to bring it back to normal. Contrary

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E. A. Ramskill, Naval Research Laboratory, Washington, D.C. Personal communica-

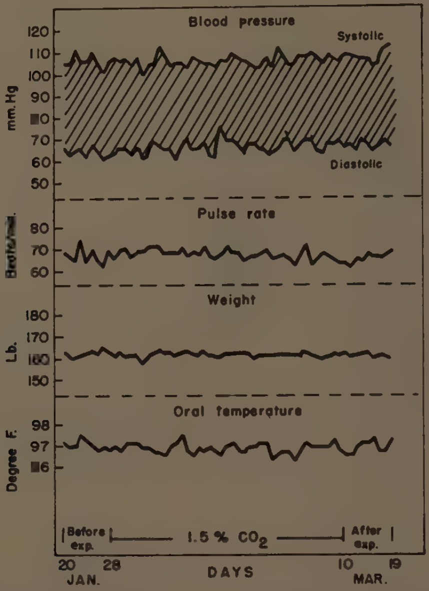


FIGURE 3. Effect of prolonged exposure to 1.5 per cent CO_2 over a period of 42 day systolic and diastolic blood pressure, pulse rate, body weight, and oral temperature (r values of 23 subjects). (Reproduced by permission of *Aerospace Medicine*.)

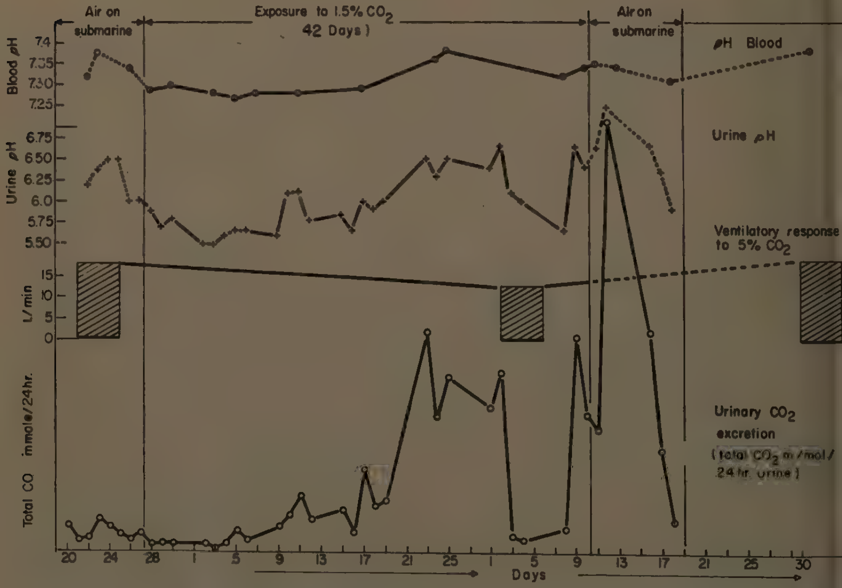
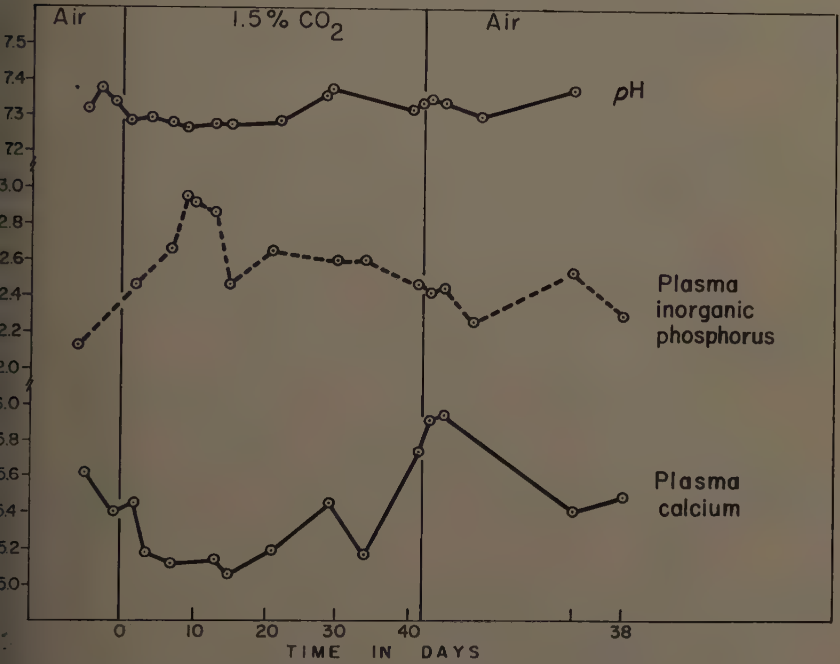


FIGURE 4. Effect of prolonged exposure to 1.5 per cent CO_2 over a period of 42 day pH of blood and urine, CO_2 excretion in the urine, and ventilatory response to 5 per cent (21 subjects). (Reproduced by permission of *Aerospace Medicine*.)

xpectation, a further drop in respiratory rate occurs, and the alveolar pCO_2 l is maintained at an elevated point for 9 days after return to air breathing URE 6). We therefore have two periods: (1) the compensated phase of iratory acidosis from the 24th day to the 42nd day of exposure; and (2) 9-day recovery period on air following exposure to CO_2, in which the pH ractically normal and the pCO_2 is elevated, while the blood pH changes limited to the first 23 days of exposure.

t therefore appears possible to delineate those functions that are particularly uenced by the pH changes of the blood from those that seem to be effected



IGURE 5. Effect of prolonged exposure to 1.5 per cent CO_2 over a period of 42 days on pH ood plasma, inorganic phosphorus, and plasma calcium.

lominately from pCO_2 changes. An example of pH-dependent variables een in the changes of plasma calcium that mirror the alterations in blood (FIGURE 5). The plasma inorganic phosphorus shows the opposite trend. IGURE 6 shows some other physiological parameters that appear to be pendent of pH changes or that, still better, are not influenced by the return lood pH to normal. This is the pattern of respiration already mentioned ve. The elevated pCO_2 seems to be the dominant factor in the development of a large tidal volume and a small respiratory rate, a pattern that in maintains an elevated pCO_2 as seen in the recovery period.

he cardiovascular capacity was tested with the Harvard step-up test, isting of stepping 20 times within 30 sec. on an 18-inch high stool. Pulse rate was counted immediately after the test, and 2 min. later. Cardiovascu score was computed as follows:

15 sec. to 20 sec. pulse rate plus
(1 min. 45 sec. to 2 min. 15 sec. pulse rate)

It can be noted that the cardiovascular score increases throughout the expos to CO_2, indicating a reduction in cardiovascular capacity. The cardiovascu score continues to rise after the compensation of respiratory acidosis is reach

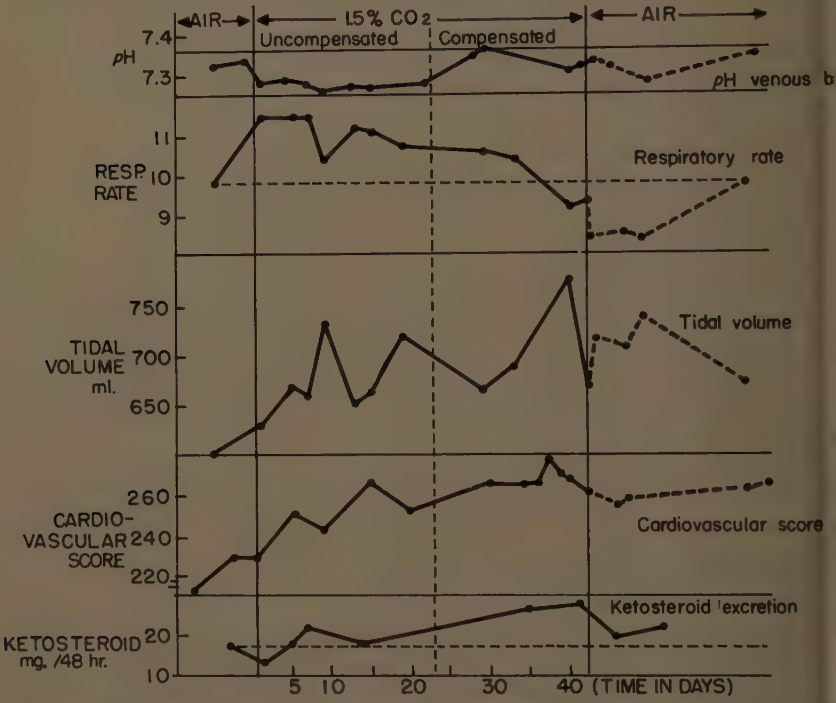


FIGURE 6. Effect of prolonged exposure to 1.5 per cent CO_2 over a period of 42 day respiratory rate, tidal volume, cardiovascular score, and ketosteroid excretion (21 subje

and remains high in the recovery phase associated with the elevated *p* level.[8]

As a measure of the stress of CO_2 exposure, the ketosteroid excretion[9] measured and found higher in the compensated phase of respiratory acid The eosinophils also began to fall during this second phase, reaching the low value during the 9-day recovery period.[9] It could be argued that the fa of confinement might have caused these effects, rather than the elevated *p* levels. However, the results of animal experiments carried out simultaneou under the same conditions of exposure to 1.5 per cent CO_2 on rats and gui pigs showed similar findings, a significant increase in adrenal-cortical acti during the same two periods in which the *p*H was not different from con levels and the pCO_2 elevated.[10]

Effects of Alkalinizing Agents in Chronic Respiratory Acidosis Induced by Prolonged Exposure to 2 to 3.5 per cent CO_2

Гhese experiments were carried out by R. Pointner.[11] Some of these data individual subjects were summarized and statistically evaluated, and are ›orted here. The subjects were exposed for 4 days to 2 to 3.5 per cent carbon ›xide. There was a control period on air lasting for 2 to 3 days prior to ›osure, and a 3-day recovery period on air following exposure. Measure-nts were made during these 3 periods of urinary *p*H and of excretion of CO_2 l ammonia; determinations were also made of intake and excretion of sodium, tassium, chloride, phosphorus, calcium, and water.

ΓABLE 1 shows the effects of these alkalinizing agents on urinary *p*H, CO_2, l ammonia excretion prior, during, and after chronic respiratory acidosis

TABLE 1*

ꟻECT OF ALKALINIZING AGENTS† ON URINARY *p*H, CO_2, AND AMMONIA EXCRETION PRIOR, DURING, AND AFTER CHRONIC RESPIRATORY ACIDOSIS (FOUR SUBJECTS)

		*p*H			CO_2 (total) gm.			Ammonia		
		———	Na salt	K salt	———	Na salt	K salt	———	Na salt	K salt
ntrol period on air to 3 days	Mean	6.36	6.25	6.30	5.95	6.4	13.5	623	663	555
	SD	0.17	0.07	0.42	1.2	0.36	13.3	64	109.5	48.8
	N	4	2	2	4	2	2	4	2	2
›osure to 2 to 3.5% CO_2 for 4 days	Mean	6.14	6.81‡	6.90	7.7	38.7§	79.0‡	741	443	461
	SD	1.14	0.09	0.00	2.03	0.64	7.3	156	15.6	14.9
	N	4	2	2	4	2	2	4	2	2
covery period on ir following CO_2 xposure 3 days	Mean	6.35	6.51	6.58	8.6	15.5	26.3	528	685	436
	SD	0.14	0.44	0.02	4.5	5.1	4.1	99	35.8	17.7
	N	4	2	2	4	2	2	4	2	2

* Adapted from Pointner.[11]

† Na salt (Sepdelen 7) and potassium salt.

‡ Values significantly different from control values at the 5 per cent level and better.

§ Values significantly different from control values at the 1 per cent level and better.

nbined with the results of an experiment in which no medication was used. BLE 2 gives some information about the retention of electrolytes based on difference between intake and urinary excretion. Unfortunately no meas-ments of electrolytes in feces were made, consequently no complete balance ıld be established.*

Гhe sodium salt Sepdelen 7 normally has diuretic effects that were not sent under conditions of CO_2 exposure in which a water retention was found BLE 1). There was an increased chloride and potassium excretion under $_2$ exposure, but no significant change in sodium excretion. Alkalinizing ›cts of sodium salt medication were shown in an increase of *p*H and the CO_2 retion of the urine and a decrease of the ammonia excretion. The results

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* The potassium phosphorus salt consisted of two thirds potassium carbonate and one l potassium biphosphate; the sodium salt (Sepdelen 7) consisted of 22 per cent sodium ıte, 23 per cent sodium tartrate, 26 per cent sodium sulphate, 25 per cent secondary sodium sphate, and 4 per cent sodium carbonate.

TABLE 2*

EFFECT OF ALKALINIZING AGENTS† ON THE EXCHANGE OF H_2O, K, Na, Cl, AS INDICATED IN THE DIFFERENCE BETWEEN INTAKE (FOOD) AND URINARY EXCRETION

		H_2O			K			Na			Cl		
		----	Na salt	K salt	----	Na salt	K salt	----	Na salt	K salt	----	Na salt	K salt
Control period on air 2 to 3 days	Mean	322	270	680	3.01	3.86	1.40	0.36	0.38	0.46	−0.412	−0.21	−1.08
	SD	199	127	67	0.86	0.51	1.01	0.46	0.88	0.10	0.32	0.97	1.31
	N	4	2	2	4	2	2	4	2	2	4	2	2
Exposure to 2 to 3.5% CO_2 for 4 days	Mean	600	580	700	1.93	0.07	2.57	1.83	0.88	1.00	0.29	3.13	0.254
	SD	183	169	149	1.04	0.37	0.15	0.73	1.3	0.06	0.70	4.13	0.18
	N	4	2	2	4	2	2	4	2	2	4	2	2
Recovery period on air following CO_2 exposure 4 days	Mean	555	200	1010	2.42	1.05	1.39	2.11‡	−0.90	1.8	1.92‡	1.19	1.40
	SD	136	297	113	1.92	0.67	0.96	0.64	1.1	1.24	0.51	3.03	0.10
	N	4	2	2	4	2	2	4	2	2	4	2	2

* Adapted from Pointner.[11]

† Na salt (Sepdelen 7) and potassium salt.

‡ Values significantly different from control values at the 5 per cent level and better.

potassium salt medication were somewhat similar to those of sodium salt atment. The normally diuretic effect of potassium salt was not maintained ring CO_2 exposure. Chloride excretion was reduced, and a slight increase retention of potassium and sodium was noted. The alkalinizing effect was monstrated in the increase of pH and CO_2 in the urine, as well as in the crease of ammonia excretion.

The CO_2 dissociation curves of whole blood were taken under the same perimental conditions. Venous blood was equilibrated with 30, 40, and 50

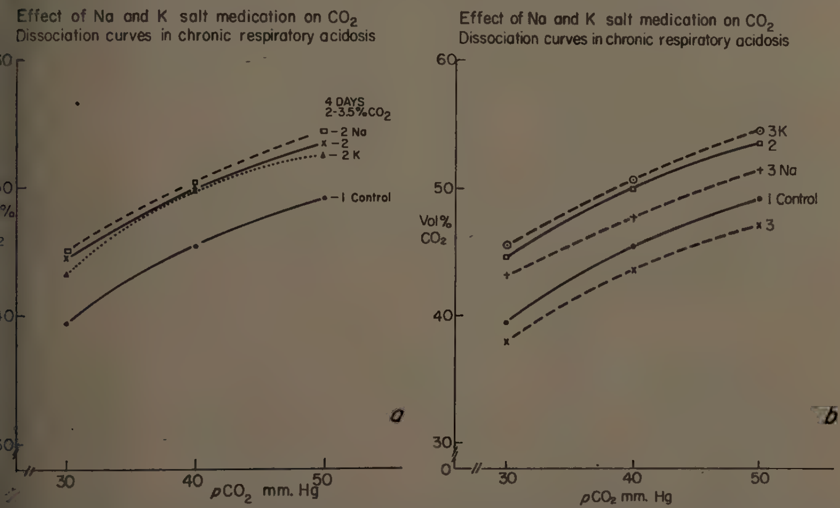

FIGURE 7(a). Effect of alkalinizing agents (Na salt and K salt) on CO_2 dissociation curves hronic respiratory acidosis. Key: (1) control; (2) 4 days, 2 to 3.5 per cent CO_2, (2Na) as , plus medication of Na salt, 10 gm. daily; (2K) as in 2, plus medication of K salt, 10 gm. ly.

b). Effect of alkalinizing agents (Na salt and K salt) on CO_2 dissociation curves in respiory acidosis. Key: (1) control; (2) 4 days 2 to 3.5 per cent CO_2, (3) 2 hours, 5.5 to 6.0 per t CO_2, following exposure to 2 to 3.5 per cent CO_2 for 4 days (1 to 3, 4 subjects); (3 Na) as , plus medication of Na salt, 10 gm. daily; (3K) as in 3, plus medication of K salt, 10 gm. y (3 Na and 3 K, 2 subjects). (Adapted from Pointner.[11])

1. Hg CO_2 tension in tonometers at 37° C. The results are shown in FIGURES and b. The well-known increase in the buffer capacity of blood in chronic piratory acidosis is demonstrated in the elevation of the CO_2 dissociation ves (No. 2, FIGURES 7a and b). Medication of Na salt or K salt did not duce a further rise in the CO_2 dissociation curves after 5 days of exposure 2 to 3.5 per cent CO_2 (FIGURE 7a). However, the subjects who had received fer salts appeared to be more resistant to a 2-hour exposure of 5.5 to 6.0 cent CO_2, following prolonged exposure to 2 to 3.5 per cent CO_2 for 5 rs. Under these conditions, a fall of the CO_2 dissociation curves occurs . 3, FIGURE 7b) that is completely prevented by K salt treatment and uced by Na salt medication (No. 3 K and 3 Na, FIGURE 7b).

urthermore beneficial effects, particularly of potassium buffer salts, were noted in the alleviation of symptoms (such as headaches, restlessness, and flig of thought) that developed during the initial phase commensurate with uncompensated respiratory acidosis. However, symptoms associated w the second phase (compensated respiratory acidosis[12]), such as a general dep sion and apathy, did not seem to be relieved by potassium salt medicatio

These findings, under conditions of a moderate chronic respiratory acid induced by a 4-day exposure to 2 to 3.5 per cent CO_2, indicate that oral med tion of alkali salts does not raise the bicarbonate CO_2 level in the blood ab a value reached by acid-base regulating mechanisms alone. Additional alk however, must be stored in tissues and made available during an acute expos to a higher CO_2 concentration of 5.5 to 6 per cent CO_2 following chronic ex sure to 2 to 3.5 per cent CO_2. This interpretation is in line with the findi of Swan *et al.*,[13] who demonstrated in nephrectomized dogs that 74 per cen the infused bicarbonate was buffered from the tissues. The beneficial eff of these drugs in alleviating symptoms associated with the acute phase respiratory acidosis are probably related to a more rapid restoration of blood pH to normal. Compensation of the pH is accomplished without dr within 3 days during exposure to 3 per cent CO_2.[5] Symptoms persist during the chronic compensated phase of respiratory acidosis due to increa pCO_2 are apparently not influenced by oral alkali medications.

Discussion and Conclusions

In the described experiment with 1.5 per cent CO_2 a combination of resp tory and metabolic processes occur. A plot of the obtained pH and bi bonate data shows that the values lie above the normal buffer line, indicat a combination of respiratory acidosis with a metabolic alkalosis, the la due to renal compensation.

The question also might be raised whether a potassium deficiency devel during the compensated phase of respiratory acidosis secondary to an increa HCO_3^- excretion. If this had been the case, a decrease in the plasma K le should have developed during the 18-day period of exposure in which the H excretion was markedly increased. However, the venous plasma potassi level showed no significant decrease.

Data on blood pH and pCO_2 homeostasis in chronic respiratory acid induced by prolonged exposure to 1.5 per cent CO_2 were shown to be rela to specific changes in physiological functions. The significance of these est lished relationships depends to a certain extent on whether changes in bl pH produce corresponding changes in intracellular pH of tissues under th conditions, as is generally assumed, since no direct measurements on in cellular pH were obtained. There is evidence from studies on isolated tiss such as muscle that the intracellular pH is influenced very little by pH char in the blood produced in acute experiments but is affected to a greater ext by changes in carbon dioxide tension of blood.[14]

These findings obtained with the DMO method are in agreement with ot based on the CO_2 method of measuring intracellular pH.[15,16] E. D. R (elsewhere in these pages), using the DMO method for the determinatio whole body intracellular pH determination in acute experiments with d nonstrated that CO_2 administration resulted in parallel decreases of extra-lular (blood) pH and intracellular whole body pH, while bicarbonate infusion l not produce corresponding intracellular pH changes.

Some preliminary results obtained with the CO_2 method in intracellular pH liver and muscle of guinea pigs exposed for periods up to 40 days to 1.5 per it CO_2 seem to indicate that, under these conditions of a slight chronic piratory acidosis, blood pH and intracellular pH show corresponding changes. rthermore, since blood pH and pCO_2 are generally used as indicators for ine therapy in chronic respiratory acidosis, the response of physiological

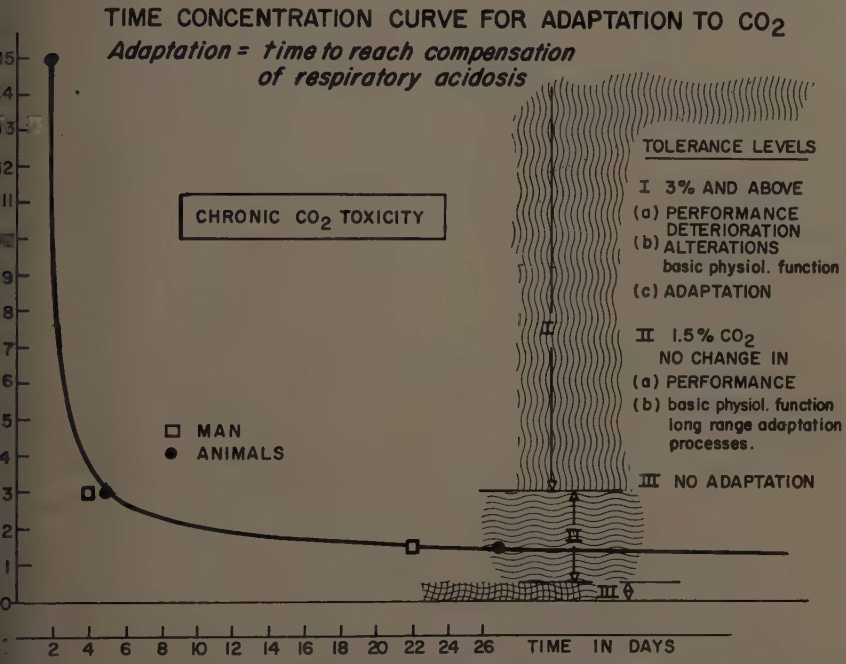

FIGURE 8. Time concentration relationship in adaptation to increased CO_2 concentration ed on experiments in humans and animals and tolerance limits for chronic CO_2 toxicity ed on three different levels of activity. (Reproduced by permission of *Aerospace Medicine*.)

ctions to slow changes in blood pH and pCO_2 are of importance and can e some further leads in the evaluation of amine effects.

Evidence attesting to effects of increased pCO_2 independent of pH changes us to the opinion that beneficial effects of amines and other buffers are ited to the correction of pH changes. It is suggested by G. G. Nahas ewhere in this monograph) that THAM might combat fatigue, since it etrates into the muscle and reduces acidity. In our experiments we have iced effects that belong within the general area of fatigue but are not related in increased acidity of the blood. After the pH had returned to normal and ompensation of the respiratory acidosis was reached, the persisting increased O_2 level was shown to produce a significant reduction in cardiovascular acity and an increase in physiological stress, as evidenced in a rise in keto-

steroid excretion. An effect of increased pCO_2 tension hardly could be
fluenced by amine therapy unless THAM absorbs some metabolic products t
do not affect the acidity of the blood but are produced by the increased
level.

The astonishingly long time periods required for acclimatization and de
climatization to such low CO_2 concentrations as 1.5 per cent CO_2 expres
for example, in the persisting change in respiratory pattern and altered calc
metabolism[17] suggest that some kind of pathophysiological state might dev
under these conditions. The results of animal experiments that showed
increase in kidney calcification of guinea pigs exposed to 1.5 per cent CO_2
periods of from 40 to 93 days seem to support this concept.[18] It seems dou
ful, therefore, whether long continued adaptation to slightly increased p
levels can be accomplished without altering normal physiological proces
This consideration has led to the formulation of a triple tolerance concept
chronic CO_2 toxicity,[19] which is expressed in FIGURE 8 together with a t
concentration curve for adaptation to CO_2. Time for adaptation is defi
as the time to reach a compensation of the respiratory acidosis induced
carbon dioxide inhalation. Determinations of pH, CO_2, bicarbonate lev
and electrolytes in blood and urine were used for estimations of the two ph
in respiratory acidosis. The three levels of acidosis used for tolerance li
are listed in FIGURE 8. The level at which no significant physiological,
chological, and adaptation changes probably occur is estimated to be 0.
0.8 per cent carbon dioxide in the inspired air.

Acknowledgment

I am grateful to W. E. McConnaughey,[1] Code 649B, Bureau of Ships, W
ington, D.C., for his permission to use FIGURES 1 and 2.

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QUANTITATIVE MEASUREMENT OF THE AMINE BUFFER: 2-AMINO-2-HYDROXYMETHYL-1,3-PROPANEDIOL

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Institute of Research, Walter Reed Army Medical Center, Washington, D.C.

The use of aliphatic hydroxyamines as buffers in animals and man has creat a need for a method for the precise estimation of these compounds in bo fluids. The following photometric procedure is such a method, designed use by technicians familiar with routine procedures and requiring no unus laboratory apparatus.

Principle

Basic compounds in the biological specimen (almost exclusively amin are adsorbed by Dowex-50 and thus isolated from other substances that then discarded. The amines are subsequently released from the resin by ad tion of acid, and an aliquot of the resulting solution is oxidized with potassi dichromate. The oxidation is manifested by the disappearance of the $Cr_2($ ion and appearance of the Cr^{+++} (reduced) ion. The method thus measu those compounds basic enough to be adsorbed by the resin and that also be oxidizable groups.

FIGURE 1 shows the visible and ultraviolet spectra of the $Cr_2O_7^=$ form (yello and Cr^{+++} form (green). Since the Cr^{+++} form has little absorption in t useful range of the spectrophotometer, I chose to measure the disappearar of the yellow color rather than the appearance of the green. Sensitivity creases as absorption maxima of the $Cr_2O_7^=$ form are chosen further into t ultraviolet, for example, 350 and 255 mμ. Two variations of the method w be described, the more sensitive depending on the ultraviolet absorption peal

Method I

Method I is for samples of 2-amino-2-hydroxymethyl-1,3-propanediol ran ing from 0.2 to 10 mg./ml.

Put about 1 gm. of Dowex-50, hydrogen form, 60–100 mesh, into a 50- volumetric flask. Add the sample (1 to 5 ml.) and enough water to make t total 5 ml. Swirl for about 3 min., then add water up to the neck of t flask, and place the flask into an approximately 45° position for a few minu until the resin has completely settled, tapping the flask if necessary. It necessary to fill the flask to the neck, since a few particles of the resin always float, due to unwettability: these particles will fall to the bottom o when the level of water reaches a point where its surface area is suddenly creased. Hold the flask in an almost horizontal position and carefully deca the water, without losing resin (it may be necessary to touch the water emergi at the opening with a glass rod in order to avoid turbulent flow). Add abo 10 ml. more water, swirl, make this up to the neck, and decant again. Th add 5 ml. of 6 *N* H_2SO_4, swirl for 2 min., make to the mark with distilled wat and shake. Allow the resin to settle to the bottom of the flask, and pipett

. of the solution into a colorimeter tube (18 × 150 mm.). Add 3 ml. di-
romate solution (see below), shake well, cap the tubes loosely (we use chil-
n's marbles) and put into a boiling water bath for 30 min. Allow to cool.
e rest of the procedure is described as done with the Bausch and Lomb
pectronic 20.” The “blank” (water or control sample run through the
ire procedure) is set to read 0.7 optical density (O.D.) at 450 mμ. Those
nples with the greater amount of the hydroxyamine will be less yellow and
l read less than 0.7 O.D. To make a calibration curve, start with a standard

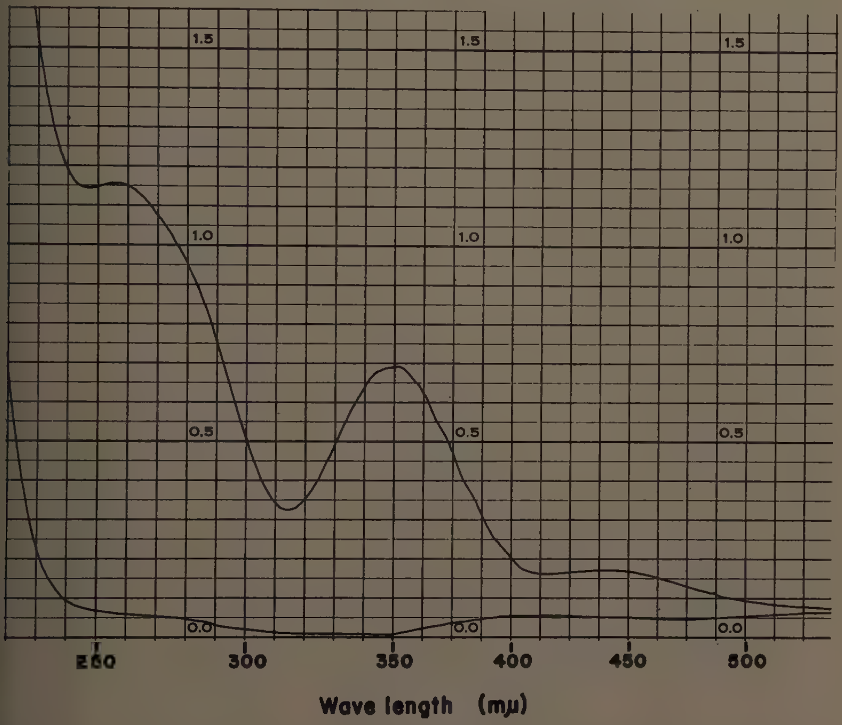


FIGURE 1. Absorption spectra of $Cr_2O_7^{=}$ (upper trace), and Cr^{+++}, both in concentrated
l.

ution of 10 mg./ml. Pipette aliquots of 0.5, 1, 2, 3, and 4 ml. into 50-ml.
sks, and follow the procedure as above. Line **1** in FIGURE 2 shows a typical
ibration at 450 mμ. 2-Amino-2-hydroxymethyl-1,3-propanediol, added to
man urine, is recovered in yields of 95 to 100 per cent when method I is used.
n consecutive analyses performed on the same urine specimen were identical
within 1.5 per cent.

The reagents are prepared as follows:

Dichromate: 1 gm. of potassium dichromate in 500 ml. of 20 *N* sulfuric acid.

Dowex-50 × 8: The mesh size of the resin is rather important; it must be
ge enough to allow the particles to sink to the bottom of the flask so that the
ter solution can be decanted, but small enough to provide sufficient surface

area for efficient adsorption of the amine compound. Resin hydrogen fo
60 to 100 mesh, should be put into a beaker of distilled water and stirred vi
ously. When the bulk of the resin has settled, decant the floating fine ma
rial. Repeat several times. After washing, the resin should be dried in ai

Method II

Method II is for samples from 0.02 to 0.2 mg./ml. and requires an ultravi
spectrophotometer such as the Beckman "DU."

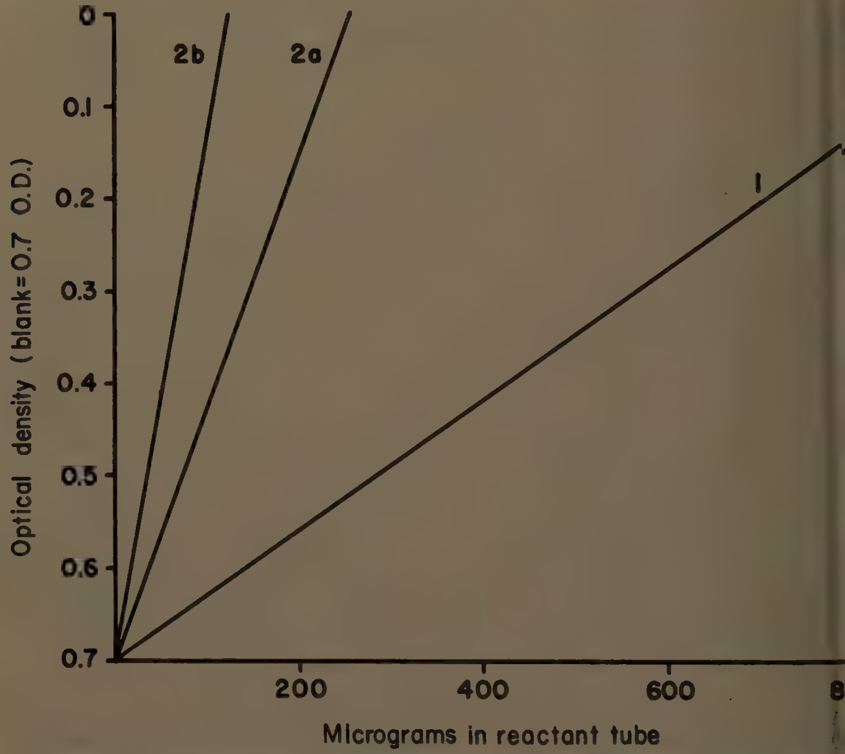
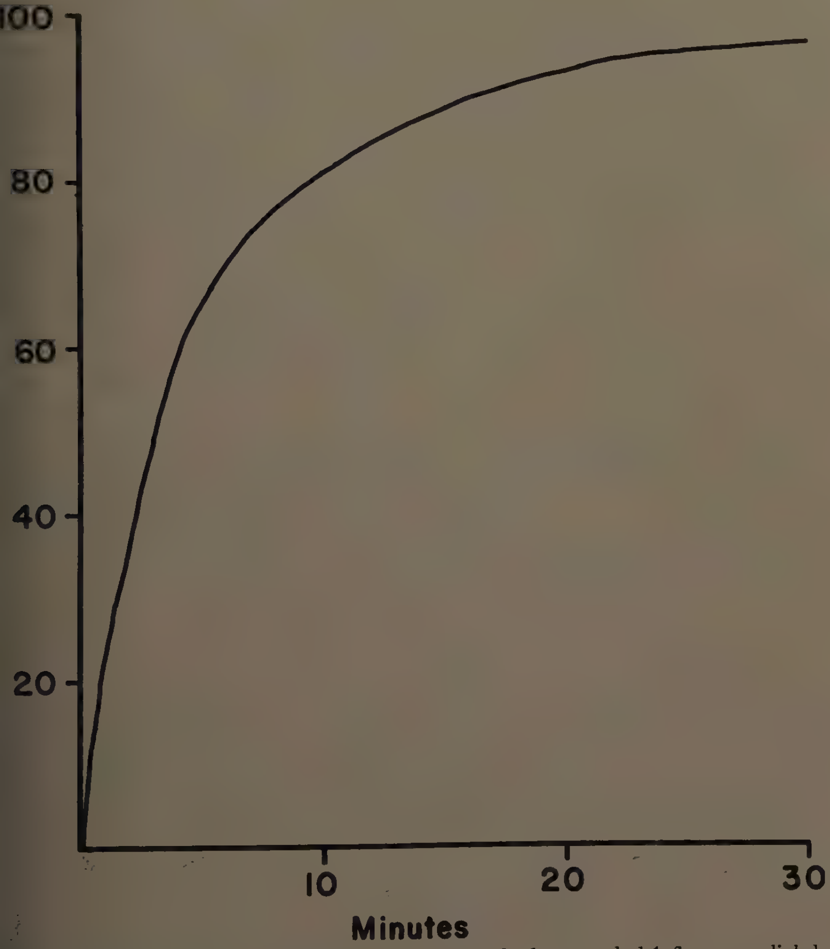


FIGURE 2. Calibration lines: **1** is obtained at 450 mμ, **2a** at 350 mμ, and **2b** at 255 m

In this modification, the dichromate solution used is 0.2 gm./500 ml. 2
sulfuric acid instead of 1.0 gm./500 ml. The final reaction mixtures, after
30-min. heating with dichromate, are placed into 1-cm. silica cells, and t
light absorption is measured in the Beckman "DU," placing the "blank"
before) at 0.7 O.D. The analysis can be carried out at 350 mμ (curve **2a**)
with even greater sensitivity at 255 mμ (curve **2b**). Appropriate standa
should be prepared.

Sensitivity and specificity. The sensitivity of the method could be increa
by a factor of at least 20 by using 10-cm.-long Beckman cells instead of 1-
cells and by making the resin suspension to a smaller volume than 50 ml.,
example, 25 or 10 ml. Such increases in sensitivity, however, would be in

ble to some biological assays because of interfering substances. For nple, approximately 95 per cent of the nonspecific reducing substances of a l. sample of normal human urine are removed by the resin. The amount in the reaction mixture, however, constitutes about 5 per cent of the apparresult if done by method I, and about 20 or 40 per cent respectively if the



IGURE 3. Time course of oxidation of 2-amino-2-hydroxymethyl-1,3-propanediol by ssium dichromate in acid solution at 100° C.

fer amine concentration is low enough to require methods IIa or IIb. Some ary compounds that might interfere with the assay (and probably constitute e of the "blank") are serine, threonine, choline, and ethanolamine. Thus n though a control sample is used for the "blank," greater purification is irable if one is to use the potentially greater sensitivity. This is quite sible if one were to employ a column of Dowex-50 or Amberlite IR-120 for ification of the amine instead of the batch method as given here.

Effect of Deviations from the Procedure as Given

(1) Heating time. FIGURE 3 shows the progress of the reaction as a funct of time, at 100° C.; 30 min. is the minimum for complete reaction.

(2) Resin. The resin particle size is rather important. It was found t the amine buffer, when shaken for 3 min. with Dowex 50, 20 to 40 mesh, adsorbed to the extent of only 15 per cent; 40 to 60 mesh resin bound 70 cent of the amine in 3 min., and 60 to 100 mesh adsorbed 100 per cent in same period. The necessity for a relatively large resin surface area is proba due to the weakly basic property of the amine buffer and consequent s exchange with H^+.

(3) Spectrophotometry. In method I, ordinary colorimeter tubes are us The effect of variations in glass quality and position is quite marked at 450 Therefore, for highest accuracy the spectrophotometer tubes should be matc and positioned in the spectrophotometer so that they face the same way e time.

(4) Glassware. Since the assay depends on the nonspecific oxidation dichromate, many contaminants will serve to reduce the reagent equally as as the amine buffer. Some wetting agents and soaps can interfere in this w For this reason, glassware should be well rinsed before use and be free of s and wetting agents.

The method has been used for the analysis of amine buffer in plasma. such an assay, the plasma must first be deproteinized with trichloroacetic (TCA), and the TCA must then be extracted with ether. The water ph can then be exposed to a stream of air while being gently heated to evapo traces of ether. The rest of the analysis is done as described.

Urine samples require no pretreatment unless proteinuria is present or pected, in which case protein must be removed as above.

During preparation of this paper, I learned from Martin Roberts* o method of estimation of 2-amino-2-hydroxymethyl-1,3-propanediol that pends on periodate oxidation and measurement of released ammonia in Con cells. This method seems comparable to the one I have described with resp to specificity, sensitivity, and reproducibility; and although it requires an o night incubation, it might be preferred by investigators familiar with estima of ammonia by the Conway technique.

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* Personal communication, Martin Roberts, Don Baxter, Inc., Glendale, Calif.

MICROASSAY OF TRIS(HYDROXYMETHYL)AMINOMETHANE APPLICABLE TO BLOOD AND URINE

Stuart Linn and Martin Roberts
Don Baxter, Inc., Glendale, Calif.

Tris(hydroxymethyl)aminomethane (Tris) has been used as a buffering agent[1] chemistry and industry for many years. It has recently received wide ention as an *in vivo* buffer for the treatment of respiratory and metabolic dosis.[2,3] No assay method for Tris in blood or urine has been published; vever, there is an unpublished colorimetric method by H. Rosen* (personal nmunication) that involves the separation of Tris by ion exchange and re-ion with acid dichromate.

The method described below is an adaptation of a serine assay[4] and depends on the quantitative oxidation of Tris to ammonia by alkaline periodate. e assay is suitable for samples containing 0.2 to 1.3 mg. Tris, and can be dily modified to obtain greater sensitivity.[5]

METHODS AND MATERIALS

Apparatus

Conway microdiffusion dish. Cells† employing liquid seals[6] were used.

Aeration device for urine samples. Air was washed with 3.6 N sulfuric acid d then bubbled through the alkalinized urine to remove ammonia. A device ilar to that shown by Hawk[7] was suitable.

Wintrobe hematocrit tubes. Tubes† of this type were used for blood samples.

Pasteur pipettes. Disposable pipettes† were used.

REAGENTS

General

Hydrochloric acid (0.01 N). The acid was standardized after a few drops of following indicator were added: 0.08 gm. of methyl red and 0.02 gm. of thylene blue, dissolved in 100 ml. of 1:1 alcohol-water solution and adjusted a neutral gray color with 0.01 N sodium hydroxide.

Sodium hydroxide (0.01 N). A carbonate-free solution was prepared and ndardized with the 0.01 N HCl.

Periodic acid (0.05 M). $HIO_4 \cdot 2H_2O$, 1.14 gm., was dissolved in 100 ml. monia-free water.

Saturated potassium metaborate solution. Boric acid, 371.4 gm., and 336.6 gm. potassium hydroxide were mixed and then dissolved by slowly adding 720 ammonia-free water. One should be cautioned that heat of reaction may se boiling.

Tris(hydroxymethyl)aminomethane. Recrystallized Tris (Sigma 121)‡ was d.

Walter Reed Army Institute of Research, Washington, D.C.
Purchased from Scientific Products, Evanston, Ill.
Purchased from Sigma Chemical Company, St. Louis, Mo.

Twenty per cent trichloroacetic acid (*TCA*). Twenty gm. of this compou was dissolved in 100 ml. ammonia-free water.

Silicone antifoam. Dow Corning Antifoam B* was used.

Procedure for Assay

A sample containing approximately 1 mg. of Tris per 0.5 ml. or less w pipetted into the outer chamber of the Conway Cell. Two ml. of the 0.01 acid was placed in the central chamber of the cell. Three ml. of saturat potassium metaborate buffer was added to the Tris and mixed by rocking t cell. Metaborate buffer was placed in the well provided for the liquid se Finally, with an eyedropper, approximately 1 ml. of periodic acid was added the Tris-buffer mixture and the cell was immediately sealed. The cell w allowed to stand overnight.[5] The acid in the central chamber was then titrat to a neutral gray color with the 0.01 N base. A blank, using distilled wa in place of periodic acid, served as a correction for any contaminating ammon

Preparation of Blood Samples

The blood sample was collected in a tube containing about 50 U. of hepar An exact amount of the heparinized blood (300 μl. to 1000 μl.)† was pipett into a clean, dry tube. An equal amount of the TCA solution was added a the precipitate was dispersed by shaking the tube. With a Pasteur pipe the well-mixed sample was transferred to a hematocrit tube and centrifuged 15 min. The levels of the solid and liquid phases were recorded. By means a Pasteur pipette, the supernatant was removed and an exact amount (300 to 500 μl.) of the supernatant containing about 1 mg. of Tris was pipetted ir the outer well of the Conway Cell. The previously mentioned procedure completing the assay was followed.

Preparation of Urine Samples

Four ml. of urine and 3 ml. of 20 per cent TCA were placed in a centrifu tube and centrifuged for 15 min. Three ml. of the supernatant and 10 ml. the buffer solution were placed in a 25 ml. tube. One drop of antifoam w added and the solution was aerated for 45 min. to remove ammonia. amount of the sample containing approximately 1 mg. of Tris was pipett into the outside well of the cell and the previously mentioned procedure completion of the assay was followed. If necessary, urine samples less thar ml. can be used.

Calculations

$$\frac{\text{mg. Tris}}{\text{ml. solution}} = \left(\frac{2NA}{NB} - m\right) \times \frac{NB}{S} \times 121.14$$

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* Purchased from Dow Corning Corporation, Midland, Mich.

† More than 1 ml. of blood can be used if available. A calibrated centrifuge tube is u instead of a hematocrit tube.

$$\frac{\text{mg. Tris}}{\text{ml. blood}} = \left(\frac{2NA}{NB} - m\right) \times \frac{NB}{S} \times 121.14 \times 2 \times \frac{VT - 0.5VP}{VT}$$

$$\frac{\text{mg. Tris}}{\text{ml. urine}} = \left(\frac{2NA}{NB} - m\right) \times \frac{NB}{S} \times 121.14 \times \frac{7}{4} \times \frac{13}{3}$$

ere m = ml. of sodium hydroxide required for titration
B = normality of the sodium hydroxide
A = normality of the hydrochloric acid
T = volume of total substance in the hematocrit tube
P = volume of the precipitate in the hematocrit tube
S = volume of sample placed in the Conway Cell.

$$\frac{VT - 0.5VP}{VT}$$

resents a correction for the volume of solid. It has been found (12 trials) t after centrifuging for 15 min. at 850 g (3600 rpm with an International

TABLE 1
ASSAY OF KNOWN AMOUNTS OF TRIS IN BLOOD

Actual Tris concentration (mg./ml.)	Measured Tris concentration (mg./ml.)	Error (%)
1.333	1.357	+1.8
1.333	1.360	+2.0
1.983	2.033	+2.5
1.983	2.007	+1.2
1.983	2.010	+1.4
1.983	1.970	−0.7

nical Model Centrifuge) the precipitate contained 50 per cent ± 4 per cent d. This value will of course be altered by different centrifugation condi- ns.

RESULTS

lood samples not containing Tris were assayed. The average apparent s concentration (7 values) was 0.060 mg. Tris/ml. Corrections for free monia (4 values) averaged 0.017 mg. Tris/ml. Thus interfering compounds vide an apparent concentration of 0.043 mg. Tris/ml. blood.

lood samples containing known amounts of Tris were then assayed (TABLE The average error was +1.5 per cent with a standard deviation of 0.7 per t. Using the blank correction for free ammonia, the average error was .5 per cent.

rine samples known to be free of Tris were assayed. As the free ammonia l already been removed, no blank correction was necessary. The average arent concentration (9 values) was 0.090 mg. Tris/ml. due to interfering npounds.

Known amounts of Tris in urine were assayed (TABLE 2). The average er was 0.1 per cent with a standard deviation of 1.2 per cent. The addition of mg. of urea and 500 units of heparin caused no interference in the assay.

The method was used in the following experiment. A rabbit was infus with 7300 mg. of Tris for 5 hours, followed by normal saline for 2 hours. Blo and urine samples were assayed (FIGURES 1 and 2). As was expected, concentration of Tris in the blood and the rate of excretion of Tris in the ur rose rapidly during the infusion of Tris, then rapidly dropped off during infusion of saline. The concentration of Tris in the urine, on the other ha continued to rise during the saline infusion because of the decreased quant of urine excreted. Total recovery of Tris in the urine during the 6⅔ ho was 66 per cent.

TABLE 2
ASSAY OF KNOWN AMOUNTS OF TRIS IN URINE

Actual Tris concentration (mg./ml.)	Measured Tris concentration (mg./ml.)	Error (%)
1.15	1.14	−0.5
1.15	1.15	0.0
1.82	1.86	+2.2
1.82	1.82	0.0
1.82	1.80	−1.1

DISCUSSION

A minimum of 45 min. aeration of the urine was necessary to assure to emission of free ammonia. In addition, if the amount of TCA added was duced, disproportionately high values would result.

No attempt was made to separate the compounds responsible for slightly hi values in the blood assay, as the apparent Tris concentration of 0.043 Tris/ml. should not produce significant error in an actual assay. The er involved in assuming that the protein precipitate in the hematocit tube was per cent solid amounted to about ±0.5 per cent.

At the end of the infusion of Tris in a rabbit, 7300 mg. of Tris had be administered, and 3540 mg. of Tris had been excreted in the urine, leaving 3 mg. in the rabbit. The rabbit weighed 2.27 kg. Assuming that the remain Tris was distributed evenly in the body water (70 per cent of body weigh then the concentration would be 2.43 mg. Tris per ml. body water. This in good agreement with the measured blood concentration of 2.58 mg. Tris ml.

SUMMARY

An assay method for tris(hydroxymethyl)aminomethane in blood and urin presented. The method involves the quantitative oxidation of Tris to a monia by alkaline periodate and recovery of ammonia in a Conway mic diffusion cell. The accuracy of the method is 0.1 per cent for urine sam and 0.5 per cent for blood samples with a standard deviation of 1.2 per c

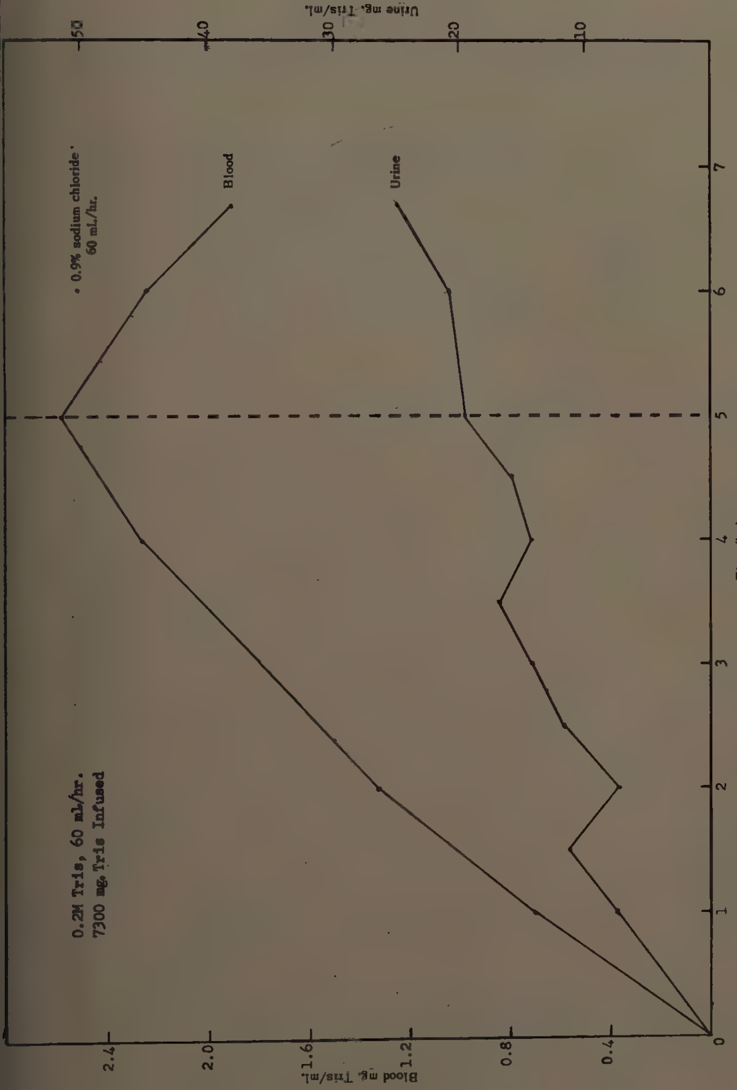

FIGURE 1. Tris concentration in rabbit urine and blood.

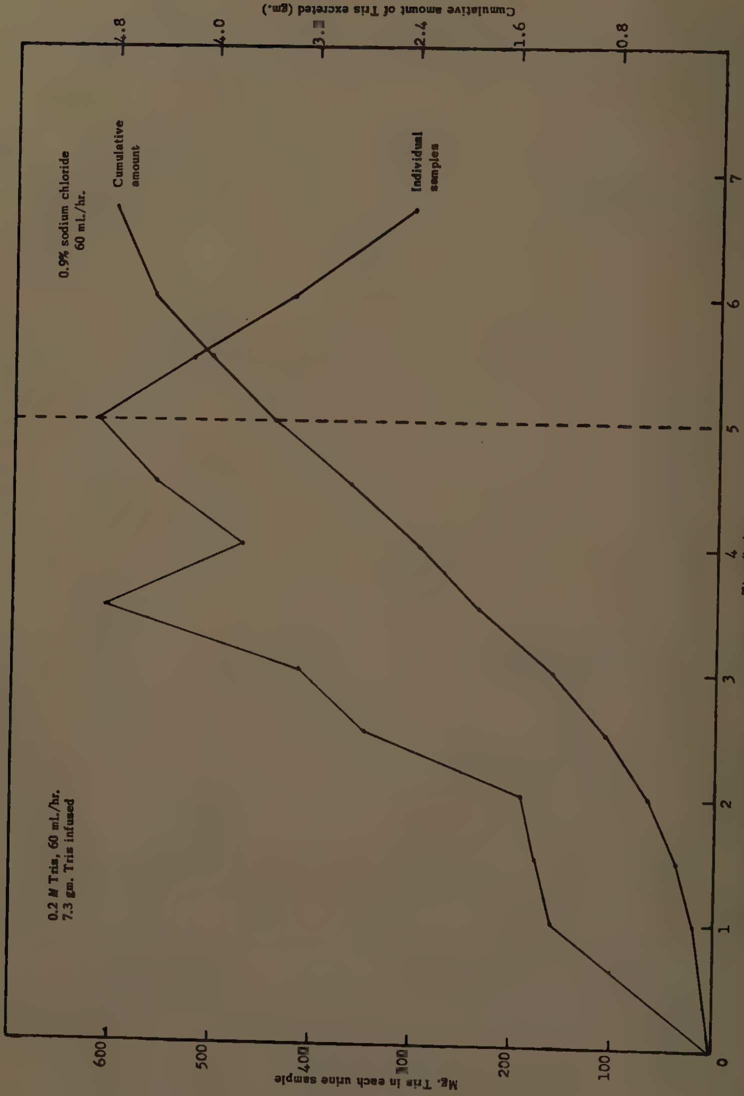

FIGURE 2. Tris excreted in urine.

0.7 per cent respectively. The method is applied to the determination of in blood and urine during and after an infusion of Tris into a rabbit.

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Part II. The Buffering of Acid Media with Amine Buffers

THE USE OF AMINE BUFFERS IN STUDIES WITH ENZYMES

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Department of Chemistry, Indiana University, Bloomington, Ind.*

Introduction

Ever since their first introduction into biochemistry by Gomori,[1] am buffers, most notably Tris (tris[hydroxymethyl]aminomethane, THAM, amino-2-hydroxymethyl-1,3-propanediol), have found favor in the eyes a variety of experimenters. The great utility of these buffers is no doubt to the fact that their conjugate acids show a favorable pK_a within the phys logical range, that their buffer regions encompass the range of pH most suita for most enzyme studies, and that their salts with appropriate acids (for ample, HCl and HOAc) constitute uni-univalent electrolytes, with the act buffer ion the conjugate acid (that is, a monovalent cation) and the base charged. Their use apparently has become so widespread in biochemist physiology, biology, and medicine that the publishing of this special monogra is warranted. Enzymologists are no exception to this trend, and a survey the recent literature discloses that Tris has become their favorite among buff Sometimes this predilection is well-founded as in the classic studies of Albe on fumarase, an enzyme that catalyzes the reaction summarized by Equatio

$$\text{fumarate} + H_2O \rightleftarrows \text{L-malate}$$

The use of Tris-acetate permitted Frieden and Alberty[2] to study this re tion throughout the pH range of from 4.0 to 10.0 with only a single buffer a with easy maintenance of ionic strength at the desired value. By compari with the results obtained in phosphate buffer, they observed a clear-cut tinction between primary pH effects on the enzyme-catalyzed reaction, on one hand and, secondary, anion effects on the other. In this manner Frie and Alberty were able to derive and test the appropriate expressions describ the behavior of the maximal velocities and Michaelis constants as a function pH and buffer ions appropriate for a reversible enzyme-catalyzed react interconverting a simple substrate and its product. The appropriate mech nisms are shown in SCHEMES 1 and 2. The reader is referred to Alberty's cussions for the analytical expressions derived on the basis of these mech nisms.[3,4]

It is, of course, implicit in this discussion that Tris is incapable of interact either with the enzymatically active site (that is, the free base, the conjug acid, and the counteranion do not function as B in SCHEME 2) or with S, substrate, or P, the product. This assumption that Tris should have no eff on the thermodynamics, the reaction path or on the enzyme in an enzy catalyzed reaction is basic to its use in enzymology. I shall show in this pa that it would be difficult to justify this assumption a priori and that ther le experimental precedent to reject it empirically, especially in view of the ligious concentration of a buffer (as compared to substrate, coenzyme, and me) in a reaction mixture. I shall examine first several possible ways in h Tris might participate in an enzymatic reaction and then cite some cases ctual examples of such participation.

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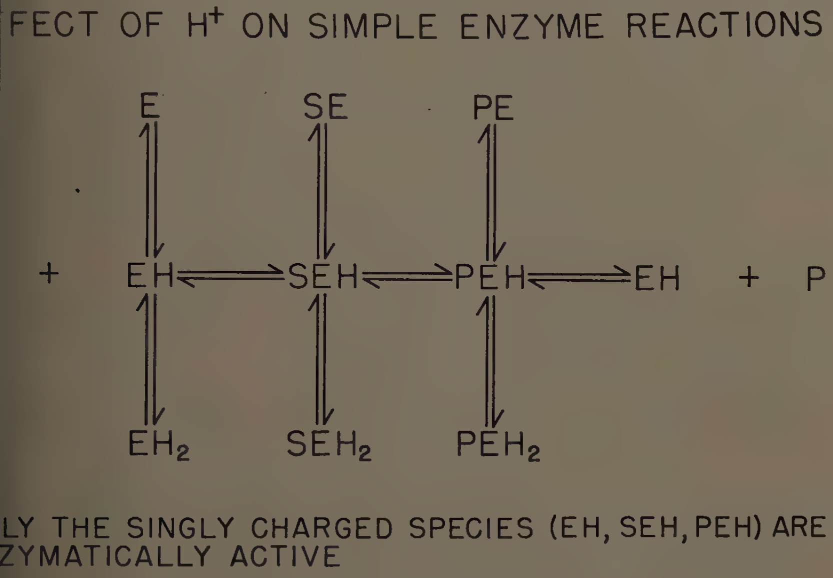
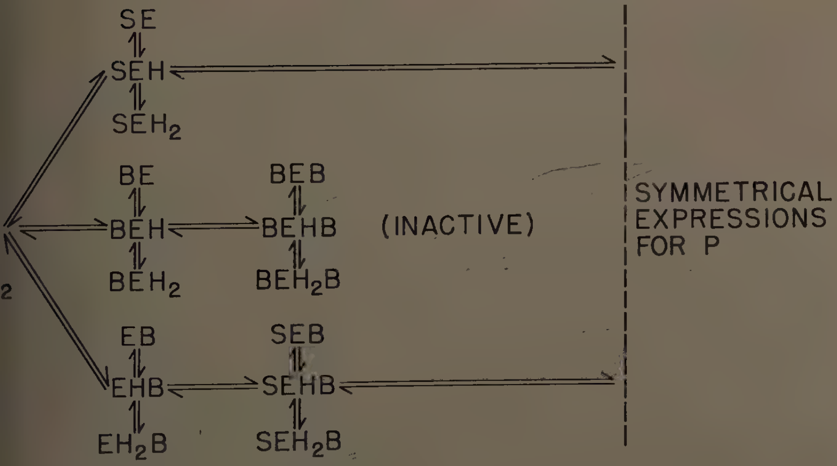
* Publication No. 978.


FECT OF H+ ON SIMPLE ENZYME REACTIONS
E
SE
PE
+
EH
SEH
PEH
EH
+
P
EH2
SEH2
PEH2
LY THE SINGLY CHARGED SPECIES (EH, SEH, PEH) ARE
ZYMATICALLY ACTIVE

Scheme 1. Buffer effects absent (Tris).



Scheme 2. Buffer effects present, including phosphate.

Possible Participation of Tris

In considering the possible roles of Tris as a potential participant in an zyme-catalyzed reaction I must of necessity start with its chemical natu The molecule contains three primary alcohol groups and one amino group, a these might be expected to affect a reaction, singly or in combination, ald one or more of the alternatives cited below.

As a possible substrate. In reactions that involve oxidation or other tra formations of carbinol groups in a molecule, the possibility exists that T might actually participate as an alternate or secondary substrate. This mig occur in those reactions in which an enzyme catalyzes the transformations primary or secondary monohydric or polyhydric alcohols (for example, glyce and glycols) or of hydroxy acids (for example, lactic, malic, glyceric, and i citric acids). An analogous situation might obtain with regard to the am group, and this possibility is to be anticipated in reactions in which ami (aliphatic or aromatic, including purines or pyrimidines) or amino acids p ticipate (for example, oxidation, deamination, transamination, condensati and acylation reactions). The situation might become especially aggrava if the actual substrate is one containing both a primary alcohol and a prima amino group, for example, ethanolamine or serine.

A special case of this general type might be expected in the case of catala This enzyme catalyzes the decomposition of hydrogen peroxide according Reaction **2**.

$$H_2O_2 \rightarrow H_2O + \tfrac{1}{2}O_2$$

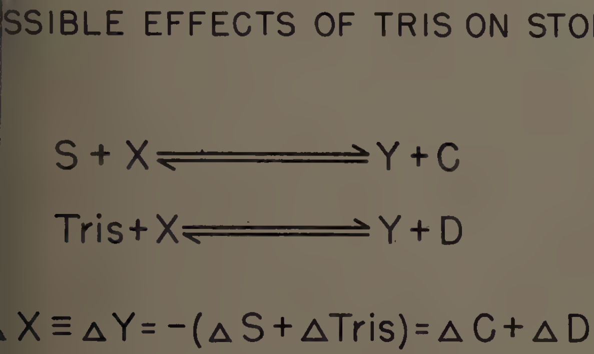
In the presence of easily oxidizable substrates such as alcohols or ami (symbolized here as SH_2), catalase is capable of the peroxidation of the lat by Reaction **3**.

$$SH_2 + H_2O_2 \rightarrow S + 2H_2O$$

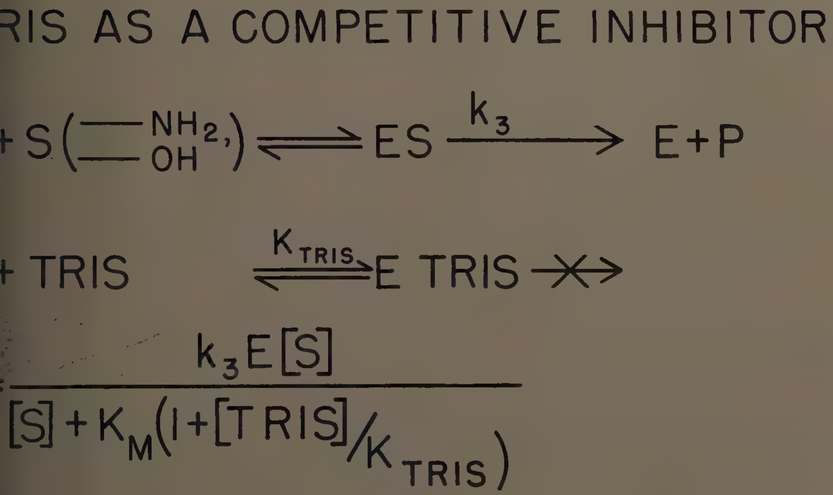
Thus the introduction of Tris might change the very path and nature o particular enzymatic reaction.

Error in stoichiometry or equilibria. If the reaction in question is measu solely by product formation and if Tris is an actual participant then it is parent that serious errors may be introduced in the measurement of the ext of reaction either in rate or in equilibrium measurements. Consider the sit tion exemplified by SCHEME 3. There the amount of X disappearing ($-\Delta$ or the amount of Y appearing (ΔY) will be stoichiometrically equivalent not $-\Delta S$ but to the sum of that entity plus $-\Delta$Tris; similarly the product form will consist of both ΔC plus ΔD (the transformation product of Tris). Ana gous considerations will govern the measurement of rates, $-dX/dt$ and $dY/$ which will not be equal to $-dS/dt$ or dC/dt.

As an inhibitor. Even if the enzyme in question is relatively specific a can discriminate between Tris and the true substrate, or if the reaction question is one which Tris is structurally incapable of undergoing (for exam the decarboxylation of an amino acid), the structural resemblance betw Tris and the substrate may still lead to important consequences. We mig find that a fraction of the enzyme molecules rather than interacting with trate might at any instant be actually engaged in a similar interaction with uffer; however, whereas the former is fruitful and leads to product forma-, the latter is not and the buffer in effect removes from the scene of action fraction of enzyme molecules so engaged and thus decreases the number of ive" enzyme molecules. The situation is that shown in SCHEME 4 where,


SSIBLE EFFECTS OF TRIS ON STOICHIOMETRIES
S + X ⇌ Y + C
Tris + X ⇌ Y + D
X ≡ ΔY = −(ΔS + ΔTris) = ΔC + ΔD

SCHEME 3.



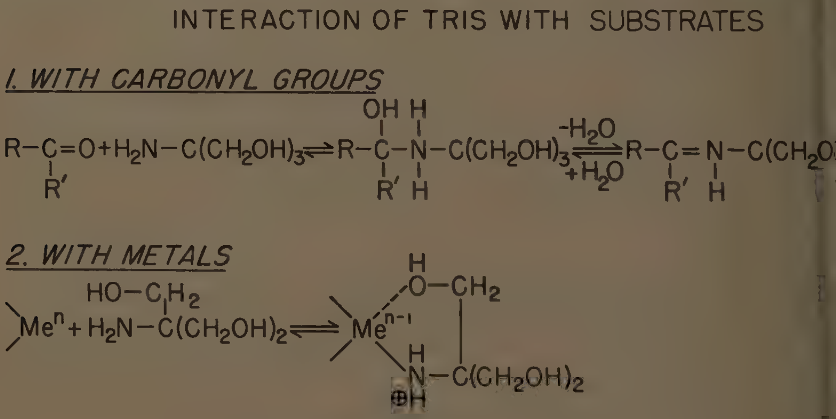
SCHEME 4.

fect, Tris is postulated as acting as a competitive inhibitor for the substrate. sideration of the kinetic law applicable to this situation discloses that, n largely by virtue of its high concentration relative to the participants in reaction, the possible rate-decreasing proclivity of Tris in a situation of this might be quite severe.

he enzymes that might conceivably be affected in this manner include, in tion to those already mentioned (that is, those catalyzing the transforma-s of unsubstituted primary alcohol and amino groups) all those that cata-the reactions in which either free or substituted primary, secondary, or tertiary alcohols or amines (including groups such as amides, esters, and p phate derivatives) or quaternary ammonium salts take part as either react or products; this is a very wide range of possible reactions indeed.

By compound formation with the substrate. The two most important mod interaction that are of concern here are reactions with carbonyl-group–cont ing and with metal-containing substrates. These are pictured in SCHEM Both interactions would have the effect of lowering the thermodynamic a ity of the substrate as well as of altering its structure; thus effects in both rate and the extent of the reactions might be anticipated. Interactions carbonyl groups may occur with substrates that are aldehydes, ketones, acids, and aldo and keto sugars.

Much less is known about the metal-containing substrates. In quite a enzymatic reactions, divalent metal ions (especially those of the alka earths and of the transition series) participate. There is now reason to



SCHEME 5.

lieve that in many instances the mechanism entails an obligatory activa of the substrate by the metal, that is, the true substrate for the reaction complex or chelate of the substrate with a metal. In such reactions an in action with Tris is a possibility. To my knowledge, actual binding stu measuring the nature and extent of actual complexation or chelating by have been performed only with Ag^+ as the metal ion. Benesch and Bene found values for log K_1 and log K_2 of 3.09 and 3.47 for the stepwise dissocia constants of Ag and 1 and 2 moles of Tris, respectively. For ethanolamin very similar molecule, the compilation by Bjerrum *et al.*[6] gives log K_1 for as about 3.1; for Hg^{++}, about 8.6; and for Sr^{++}, 1.11; the log of the ove dissociation constant for the $Cu(Tris)_4$ complex is 16.5. These values unusually high for a primary amine and indicate marked stability. several typical metals of possible significance in enzymatic reactions are bo by Tris; it might be appropriate to extend these studies to include a comprehensive and representative series of metals. Finally we must consid mode of action between Tris and substrate, which is catalytic rather than chiometric, in that some chemical (that is, nonenzymatic) transformatio ubstrate is catalyzed by the presence of Tris base and/or the conjugate because of the occurrence of either specific or general acid or base catalysis.

teractions with the enzyme or coenzyme. It might be anticipated that on sion Tris may be capable of interacting not with the substrates but with oenzymes and/or the enzymes that take part in the reaction. To mention ne specific example of such interactions, most transformations involving o acids require pyridoxal or pyridoxamine as a coenzyme. The possi-, therefore, of Tris either reacting or competing with these moieties may ppear to be too farfetched. Similar considerations may also govern pos- interactions of Tris with the catalytically active or substrate-binding on the enzyme. For instance, a large number of transferase enzymes as chymotrypsin, trypsin, thrombin, elastase, pseudocholinesterase, ali-ase, and phosphoglucomutase) have now been shown to contain serine at ctive site and to be capable of effecting and perhaps of functioning by vir-f the formation of seryl derivatives of the substrate.[7] Tris may compete this process or remove the substrate from the serine residue by transes-cation. In addition to these cases that involve primary, covalent bond ation between Tris and enzyme or Tris and coenzyme, it is also conceiva-hat Tris might affect the tertiary structure of an enzyme protein and thus ge its catalytic effectiveness by the formation of hydrogen bonds and of airs different from those present in a solution of the enzyme protein in an t" salt, such as NaCl, of the same ionic strength.

her effects. Several other properties of Tris that may on occasion be of est to enzymologists have been pointed out by some of my colleagues. ur Kornberg* cites the fact that Tris will react with periodate and thus ot be used when this useful analytical technique is to be employed. Simi-, as pointed out by D. E. Koshland, Jr.,* Tris gives a ninhydrin color, it cannot be used when amino acids are to be determined by this method. n Meister* points out that the temperature coefficient of Tris, that is, its *I* of ionization is unusually high, and therefore a great deal of variation e *p*H of any given Tris buffer is to be expected unless the temperature is ully controlled.

Specific Examples

surveying actual experimental observations that might be used in this ext I have been fortunate in having received a number of suggestions from agues. I am deeply indebted to them for their permission to use their ently unpublished data.

mpound formation with carbonyl substrates. A very startling example of type has been communicated to me by H. A. Barker.* The reaction is of pyruvate with Tris chloride at *p*H 8.6 and at either 37° C. in solution $-10°$ C. in the frozen state.† The results of this investigation are shown GURE 1. Here the concentration of pyruvate was determined spectro-

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ersonal communication.

he rates at the two temperatures are about the same, that is, ~6.7 per cent disappear- 2 hours. Barker believes that this may be due to the fact that during freezing at $-10°$ re may have been a considerably higher concentration of reactants in that part of the n mixture that froze last.

photometrically at 340 mμ by the stoichiometric conversion of reduced dip
phopyridine nucleotide (DPNH) to its oxidized form (DPN) in the presenc
lactate dehydrogenase (LDH):

$$\text{pyruvate} + H^+ + \text{DPNH} \xrightarrow{\text{LDH}} \text{DPN}^+ + \text{lactate}$$

When the reaction was measured by the well-known Friedeman-Haugen met
for pyruvate (that is, the chemical conversion of pyruvate to its dinitrophe
hydrazone, which is then measured colorimetrically) the rate of disappeara
of pyruvate in Tris appeared to be considerably slower. Barker has interpre

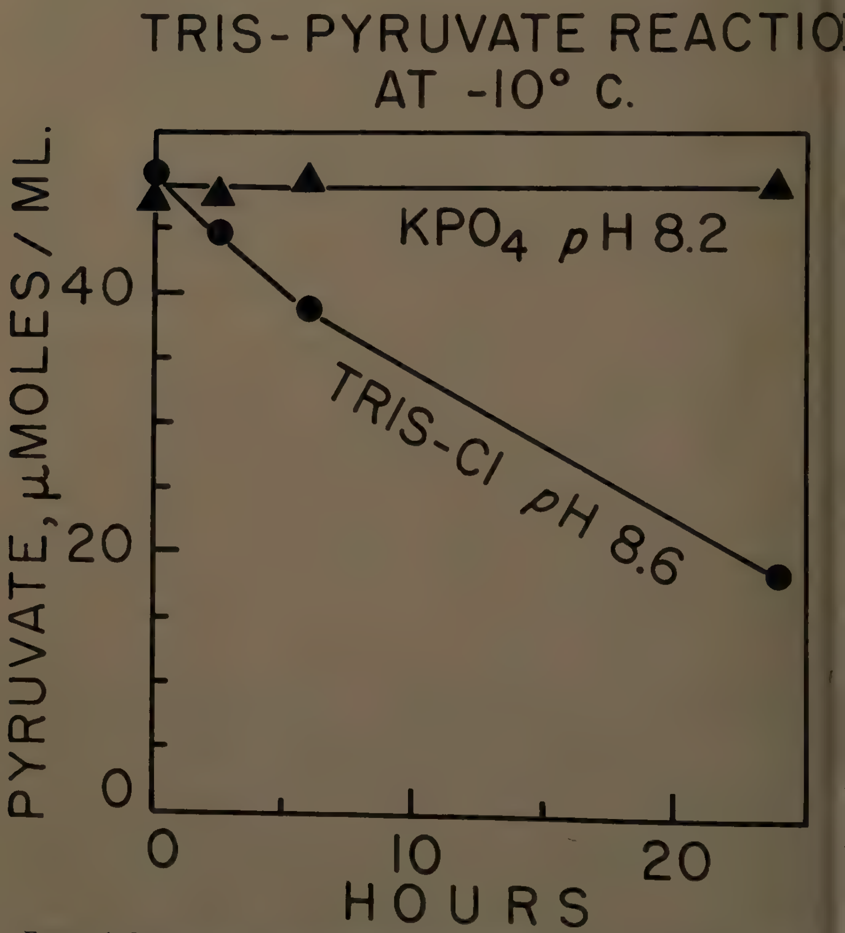
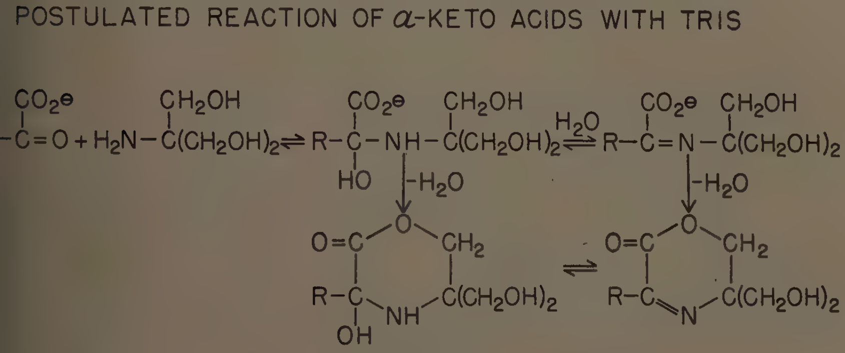


FIGURE 1. Disappearance of pyruvate in the presence of Tris. Results of an investi
tion by H. A. Barker. Concentration of buffers used was 0.10 *M*, temperature −10°
pyruvate concentration was measured enzymatically by the oxidation of DPNH with lac
dehydrogenase (LDH):

$$\text{pyruvate} + H^+ + \text{DPNH} \xrightarrow{\text{LDH}} \text{lactate} + \text{DPN}^+$$

to mean that pyruvate and Tris interact to form at least 2 compounds uentially, the first of which is inactive in Reaction **4** but is still capable of ng converted to the hydrazone, whereas subsequent ones are incapable of having like (or being converted to) pyruvate as measured by either reaction. possible sequence analogous to SCHEME 5-1 applicable in this instance is wn as SCHEME 6.

erusal of the literature shows that this reaction of Tris with a carbonyl npound probably does not constitute an isolated instance; the reaction may fairly widespread and therefore of considerable pertinence to enzymological estigations. Segal and Boyer[8] have found, for example, that either D,L-ceraldehyde-3-phosphate or D,L-glyceraldehyde in 0.01 *M* Tris, *p*H 8.4, have alf life of only 10 to 20 min. Similarly, B. L. Horecker* informs me that has some evidence for the interaction of Tris (and other buffers containing mary amino groups) with reactive ketopentose phosphate esters such as ulose phosphate or xylulose phosphate.



SCHEME 6.

ffects on the nature and extent of reaction. An interesting case of some very found effects of Tris on an important reaction is provided by the work of rtmann and Buchanan on the transformylation reaction.[9] The two reac-ns to be discussed are outlined in SCHEME 7. In the first one, the N^{10}-formyl ivative of tetrahydrofolic acid (THFA) is converted to the imidazolium ivative (5,10-methenyl-THFA), which constitutes the metabolically active m of formate. This is an equilibrium reaction that takes place in the ab-ce of any enzymic catalysis and that, as indicated by the equation, is ngly *p*H-dependent, with the imidazolium form predominating under d conditions. Not only position but the rate of attainment of the equilib-n is *p*H-dependent. At a fixed *p*H, however, the rate of interconversion of formyl and the methenyl derivative depends on the nature of the buffer. FIGURE 2, which is taken from Hartmann and Buchanan's paper,[9] the rates disappearance of the methenyl compound at 27° C. and *p*H 7.4 in 0.1 *M* sphate, Tris chloride, and in maleate buffers are compared. A possible lanation of this effect may be given in terms of general base catalysis;[10] it

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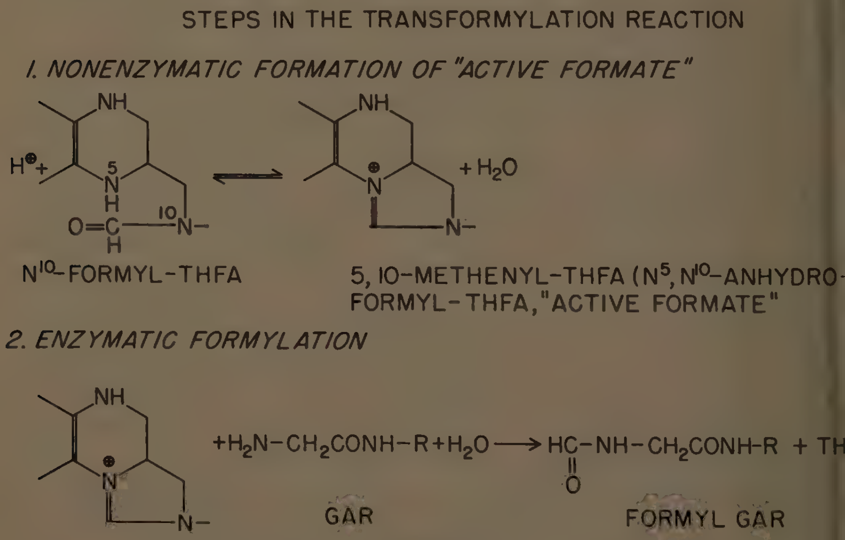
Personal communication.

is outlined in SCHEME 8. In this reaction sequence the rate-limiting ste supposed to be the buffer-dependent opening of the hydrated ring; it is enti conceivable that different buffer bases (*B*:) might well affect this step at wid differing rates.

A strong dependence on the nature of the buffer used was also reported the enzyme-catalyzed step. This is shown in TABLE 1, again taken from paper by Hartmann and Buchanan.[9] The results in the first two columns probably to be explained by the effects just discussed. Since the true form donor in the enzyme reaction is the methenyl derivative, and since its rat formation from the N^{10}-formyl THFA is strongly buffer-dependent, a sim dependence on buffer of the over-all reaction is to be anticipated. The res of the last two columns, that is, where the methenyl derivative (the true s


STEPS IN THE TRANSFORMYLATION REACTION
1. NONENZYMATIC FORMATION OF "ACTIVE FORMATE"
NH
H⊕+
5
N
H
10
O=C
N–
H
+H₂O
N⊕
N¹⁰-FORMYL-THFA
5, 10-METHENYL-THFA (N⁵, N¹⁰-ANHYDRO-
FORMYL-THFA, "ACTIVE FORMATE"
2. ENZYMATIC FORMYLATION
+H₂N–CH₂CONH–R+H₂O ⟶ HC–NH–CH₂CONH–R + TH
O
GAR
FORMYL GAR

SCHEME 7.

strate) is itself used, are not so easy to understand. Perhaps what we dealing with here is a situation similar to that postulated in SCHEME 8, tha an actual participation of the buffer in the rate-determining step of the re tion sequence.

Effects on kinetic parameters. Finally, I wish to turn to a reaction stu extensively in our laboratories* by R. H. Baker, Jr.[11] I am referring to reversible oxidation of ethanol by DPN^+ in the presence of the enzyme alc dehydrogenase that can be obtained from mammalian liver in an essenti pure form. For several months we studied this reaction, outlined in SCHEM at 27.0° C. in 0.100 *M* Tris chloride buffer, in order to benefit from the w known advantages of this buffer system. Indeed we found that the in velocity in this reaction obeyed the relationship that was first described

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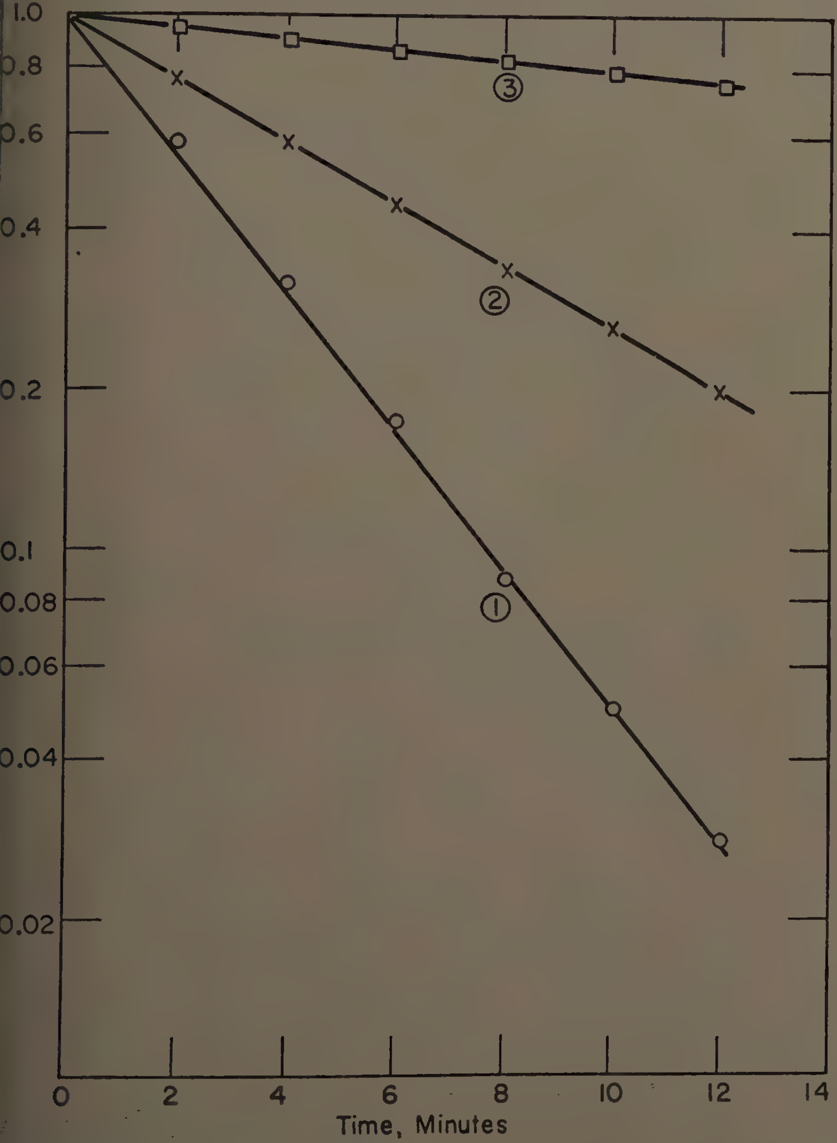
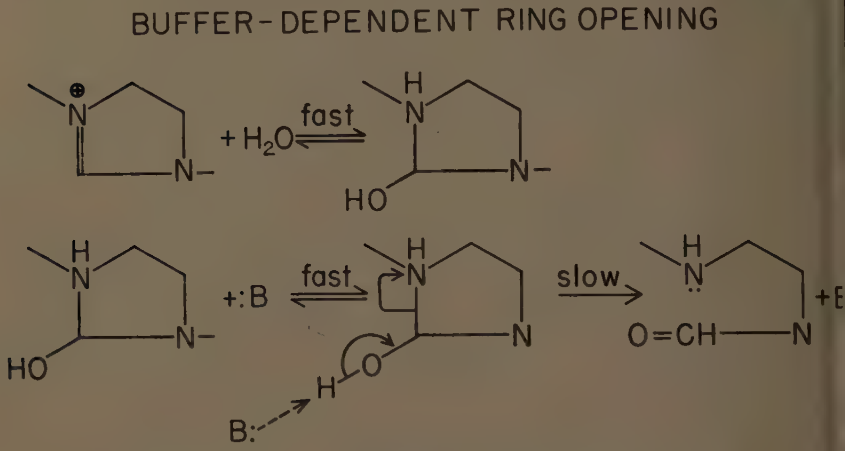
* These investigations were supported by a program grant of the American Cancer Soc New York, N.Y., and by the Office of Naval Research, Washington, D.C.

ziel,[12] and it is the two-substrate equivalent of the well-known Michaelis-
nten equation. In essence this equation states that, with one of the two
strates held at a very high concentration, the dependence of the initial
city on the concentration of the second substrate is hyperbolic (or what



IGURE 2. Effect of buffer composition on the rate of nonenzymatic conversion of N^{5},N^{10}-
droformyltetrahydrofolic acid to N^{10}-formyltetrahydrofolic acid. The absorbance at 358
s a measure of the concentration of N^{5},N^{10}-anhydroformyltetrahydrofolic acid present.
e 1, phosphate buffer; curve 2, Tris-chloride buffer; curve 3, maleate buffer. Repro-
d by permission of *The Journal of Biological Chemistry*.[9]

is equivalent, that the reciprocal of the initial velocity is directly prop tional to the reciprocal of the concentration of the second substrate). appropriate algebraic or graphical manipulation of the initial rate data—m ured in this case by the appearance or disappearance of DPNH at 340 and programmed for the solution of the rate equation on the IBM-650 compu —all the 8ϕ_i's that characterize the reaction may be determined. There



Scheme 8.

Table 1
Effect of Buffer Composition on Glycinamide Ribonucleotide Transformylase Reaction*

Buffer	Tetrahydrofolic acid formed from formyl donor (mμmoles)			
	N^{10}-formyltetrahydrofolic acid		N^5N^{10}-anhydroformyltetrahydrofolic acid	
	15 min.	30 min.	15 min.	30 min.
Maleate	0	0	2.7	5.3
Phosphate	2.5	3.7	12.1	15.5
Tris-chloride	2.1	3.1	12.4	18.1

* Reproduced by permission of *The Journal of Biological Chemistry.*[9]

certain consequences of some of the mechanisms discussed by Dalziel; important is that $\phi_{12}(H^+)/\phi'_{12}$ should equal the equilibrium constant for over-all reaction. Since this constant can be determined independently provides a good check of the reliability of the kinetic measurements. For reaction in question the equilibrium constant had been determined very curately by Bäcklin[13] (in phosphate and glycine buffers), and again by ourse in a variety of buffers. To our great chagrin, the kinetically determined v in Tris appeared to be reproducibly too high by a factor of approximate (Table 3). We tried to eliminate all possible sources of error; eventually l to face the unattractive possibility that we were dealing with some specific ect ascribable to Tris buffer itself. We therefore investigated whether Tris ght actually be a substrate for the enzyme and found this to be the case: Tris uld substitute for ethanol and was oxidized by DPN+ at a rate 0.0023 per

HE REACTION CATALYZED BY LIVER ALCOHOL DEHYDOGENASE

$$\text{ACETALDEHYDE} + \text{DPNH} + \text{H}^{\oplus} \rightleftharpoons \text{ETHANOL} + \text{DPN}^{\oplus}$$

$$E_0/V_f = \Phi_0 + \Phi_1/[\text{DPNH}]_0 + \Phi_2/[\text{ald}]_0 + \Phi_{12}/[\text{DPNH}]_0[\text{ald}]_0$$

$$E_0/V_r = \Phi'_0 + \Phi'_1/[\text{DPN}]_0 + \Phi'_2/[\text{alc}]_0 + \Phi'_{12}/[\text{DPN}]_0[\text{alc}]_0$$

$[\ ]_0 \equiv$ total concentration

SCHEME 9.

TABLE 2
COMPARISON OF KINETIC DATA

	Tris pH 7.52 ± 0.2	Imidazole pH 7.58 ± 0.2
	DPN+ + EtOH →	
ϕ'_0	$2.99 \pm 0.41 \times 10^{-1}$	$4.05 \pm 0.14 \times 10^{-1}$
ϕ'_1	$\underline{9.10} \pm 0.80 \times 10^{-6}$	$\underline{7.43} \pm 0.47 \times 10^{-6}$
ϕ'_2	$4.69 \pm 0.32 \times 10^{-4}$	$4.94 \pm 0.94 \times 10^{-4}$
ϕ'_{12}	$\underline{3.40} \pm 0.06 \times 10^{-8}$	$\underline{1.15} \pm 0.04 \times 10^{-8}$
	DPNH + ald →	
ϕ_0	$\underline{4.61} \pm 0.23 \times 10^{-2}$	$\underline{3.41} \pm 0.35 \times 10^{-2}$
ϕ_1	$\underline{3.13} \pm 0.07 \times 10^{-7}$	$\underline{1.70} \pm 0.11 \times 10^{-7}$
ϕ_2	$\underline{3.61} \pm 0.03 \times 10^{-5}$	$\underline{4.67} \pm 0.05 \times 10^{-5}$
ϕ_{12}	$\underline{5.87} \pm 0.09 \times 10^{-11}$	$\underline{2.12} \pm 0.01 \times 10^{-10}$
	DPND + ald →	
$D\phi_0$	$6.73 \pm 0.35 \times 10^{-2}$	not determined
$D\phi_1$	$3.87 \pm 0.10 \times 10^{-7}$	$3.83 \pm 0.15 \times 10^{-7}$
$D\phi_2$	$\underline{8.59} \pm 0.05 \times 10^{-5}$	$\underline{7.97} \pm 0.14 \times 10^{-5}$
$D\phi_{12}$	$\underline{1.38} \pm 0.01 \times 10^{-10}$	$\underline{2.68} \pm 0.34 \times 10^{-10}$

ignificant differences are underlined.

cent that of ethanol of the same concentration. Of course, Tris was the bu in this reaction and therefore not present at the same concentration, but rat at one 10^2 to 10^3 greater than that of the substrates used in our kinetic measu ments.

Under the conditions actually employed this would lead to an alteration the measured initial rates of only ±3 per cent. As shown in TABLE 2, howev this relatively small direct effect leads to pronounced indirect effects, presu ably ascribable to the interaction of Tris with various forms of the enzy and its function as an inhibitor of the reaction in either direction. These

TABLE 3
COMPARISON OF DERIVED CONSTANTS

Constant	Relation	Tris	Imidazole
K_{DPN}	ϕ'_{12}/ϕ'_2	7.2×10^{-5} *M*	1.6×10^{-4} *M*
k'_{on}	$1/\phi'_1$	1.1×10^5 M^{-1} sec.$^{-1}$	1.3×10^5 M^{-1} sec.$^{-1}$
k'_{off}	$\phi'_{12}/\phi'_1\phi'_2$	80 sec.$^{-1}$	21 sec.$^{-1}$
K_{DPNH}	ϕ_{12}/ϕ_2	1.6×10^{-6} *M*	4.5×10^{-5} *M*
k_{on}	$1/\phi_1$	3.2×10^6 M^{-1} sec.$^{-1}$	5.9×10^6 M^{-1} sec.$^{-1}$
k_{off}	$\phi_{12}/\phi_1\phi_2$	5.1 sec.$^{-1}$	2.7 sec.$^{-1}$
	$\phi_{12}(H^+)/\phi'_{12}$	4.8×10^{-11}	5.2×10^{-12}
K'_{eq}	dto, glycylglycine		7.4×10^{-12}
	direct measurement, glycylglycine		9.1×10^{-12}
	direct measurement[13]		1.1×10^{-11}

TABLE 4
COMPARISON OF ISOTOPE EFFECTS*

Ratio	Tris	Imidazole	Glycylglycine
K_{DPNH}/K_{DPND}	1.00 ± 0.05	1.35 ± 0.06	1.23 ± 0.14
$k^H{}_{on}/k^D{}_{on}$	1.24 ± 0.25	2.25 ± 0.23	1.71 ± 0.24
$k^H{}_{off}/k^D{}_{off}$	1.20 ± 0.58	3.05 ± 0.46	2.10 ± 0.54

* All for 0.10 *M* chlorides at 27° C.

direct effects are of importance in changing the values of the various (TABLE 2). The effects are of special importance when the values are to used for the determination of the equilibrium constants, the binding consta for the coenzymes, and the rates of association and dissociation in the inter tion of enzyme and coenzyme. I have compared the values obtained in T (where specific, substratelike interactions between enzymes and buffer occ with those in imidazole of the same *p*H (where they are presumably absent) TABLE 3. Most of our subsequent studies have been conducted in glycylg cine buffer at *p*H 8.58; thus most measurements are not directly compara One of our aims has been the determination of isotope effects occasioned by substitution of deuterium into the appropriate portion on the coenzyme. Th isotope effects should be relatively *p*H-independent, and a comparison of es obtained in Tris, imidazole, and glycylglycine (all for the chlorides and .100 *M* and 27° C.) should prove meaningful. It may be seen from TABLE at the values determined in glycylglycine (*p*H 8.58) and imidazole (*p*H) are indeed almost indistinguishable, but they differ from those measured ris (*p*H 7.52) in a significant manner. Therefore, again, measurements in have introduced a considerable error in the determination of important tic parameters.

Conclusion

ithout seeming needlessly pessimistic, I think it must have become apent from the foregoing that due caution should be exercised in the indisinate use of buffers, including primary amine buffers such as Tris in the ly of enzyme-catalyzed reactions. The effects to be anticipated range from tively trivial to quite severe and may influence the course, extent, and ure of such reactions. Tris cannot by any criterion be considered chemically er inert or unreactive in a wide variety of enzymatic reactions; its possible and influence must be examined critically in each individual case.

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AMINE AND OTHER NONBICARBONATE BUFFERS IN CELL CULTURE MEDIA*

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The application of cell-culture techniques in studying fundamental proble
in many fields of experimental biology has increased almost exponentially
the past few years. The growth of cells under standardized conditions
frequently essential to their use in many types of experiments. Traditional
cells of mammalian and avian origin have been propagated *in vitro* in me
containing sodium bicarbonate as the principal buffer. Adequate control
the *p*H of such cultures introduces a number of technical problems, for it
necessary to maintain a specified carbon dioxide tension in the atmosphere
employing gassing procedures and closed culture flasks. In an attempt
simplify the control of *p*H, a number of compounds that have the appropria
pK_a and are compatible with other ingredients of conventional media ha
been tested for their ability to replace the bicarbonate buffer.

In an earlier study[1] such compounds as tris(hydroxymethyl)aminometha
(Tris), 5,5-diethylbarbiturate (Veronal), imidazole and glycylglycine w
tested in a qualitative manner with the following cultures: human carcino
cells, strain HeLa[2] and mouse fibroblasts, strain L[3] and fibroblasts derived fr
normal human foreskin, testicles, and human embryonic kidney and lu
All cells were grown on glass in stationary cultures. The medium was co
posed of Solution 703 of Healy *et al.*[4] fortified with 5 per cent by volume of b
embryo extract (BEE) and 10 to 20 per cent horse serum (NHS). The sodiu
bicarbonate of Solution 703 (0.02 *M*) was replaced with the appropriate expe
mental buffer in concentrations varying from 0.01 to 0.05 *M* at *p*H 7.4 to 8
The modified 703 was supplemented with BEE and NHS in the same conce
trations as those employed for stock cultures.

Under these conditions veronal and imidazole are lethal for all the cells a
concentration of 0.01 *M*. On the other hand, all of the cells proliferate
media containing 0.01 to 0.02 *M* Tris or glycylglycine at a rate comparable
that of the control cultures in bicarbonate-media. Tris inhibits growth
HeLa and L at concentrations exceeding 0.03 *M*. The cultures of hum
fibroblasts are somewhat more tolerant to Tris than strains L and Hel
Frequently the response of cell cultures to an injurious agent does not beco
apparent until after prolonged exposure. This does not appear to be the c
with Tris since cells proliferate well after many months of growth in me
containing this compound at a level of 0.02 *M*. In the course of these ea
studies there were a number of indications however that Tris was inferior
bicarbonate. For example, cultures initiated with a minimal inoculum did
appear to proliferate as well in Tris as in bicarbonate. Furthermore, the c
of strains L and HeLa were more granular and had a greater tendency to fo

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and Infectious Diseases Institute, Public Health Service, Bethesda, Md.

† Research Fellow, American Cancer Society, Cuyahoga County Unit, Cleveland, Ohi

mps of cells on the glass in Tris than in bicarbonate. These data indicated
ner that Tris exerted a slight inhibitory effect on the cells which was not
entuated with time, or that a reduction in the bicarbonate level did have
ne adverse effect on the cells. The latter was shown to be the case in subse-
nt experiments.

Stock cultures of cells employed in these studies were grown in solution S77[5]
pplemented with 10 to 20 per cent NHS. S77 is similar to S103 described
lier[6] and contains the ingredients of Earle's balanced salt solution,[7] amino
ds and vitamins. The following strains were studied: strains L, HeLa,
2,[8] and Dl, recently established from the Dunning rat hepatoma.[5] In these
periments the bicarbonate (0.026 M) of S77 was replaced with 0.02 M Tris
0.02 M sodium phosphite[9] at a final pH of 7.6 to 7.8. Cells were grown in
tionary cultures[8] in stoppered flasks, and the cells were enumerated by a
clear counting procedure.[10]

The results of these experiments are presented in FIGURE 1. It will be noted
t excellent growth is obtained in media containing both Tris and phosphite,
t that in every instance the rate is somewhat less than that observed in a
dium containing bicarbonate. The rate of growth in Tris and phosphite,
wever, is restored to that of the control when 0.005 M sodium bicarbonate is
led to the medium. When loosely fitting metal caps or cotton plugs are
d instead of stoppers, the growth rate in media containing Tris and phos-
ate is significantly less than that indicated in FIGURE 1 for cells in stoppered
ks. These data indicate clearly that neither phosphite nor Tris is inhibitory
der these conditions but that the reduced rate of growth results from insuffi-
nt bicarbonate in the medium. This is further indicated by the fact that
en cultures are initiated in stoppered flasks with a large inoculum (25 to
$\times$ 10^4 cells/ml. instead of the usual 3 to 5 $\times$ 10^4 cells/ml.), growth in both
s and phosphite is comparable to that obtained in bicarbonate.

The importance of bicarbonate in regulating the rate of growth is even more
kingly demonstrated with cells proliferating rapidly in suspension (FIGURE
Strains N1S1-48 and D1S1-48 derived from the Novikoff and Dunning rat
atomas respectively[5] were grown in shake cultures in media composed of
supplemented with NHS and 0.1 per cent of the polyglycol "Pluronic
3." [11] The Pluronic F68 is not a nutrient, but permits growth in shake
tures by preventing the precipitation and inactivation of essential serum
teins. The data presented in FIGURE 2 illustrate that in a medium con-
ning 20 per cent NHS (air to liquid volume of approximately 1) the genera-
time of D1S1, in the logarithmic phase of growth, is 10 to 12 hours in bi-
bonate as compared with 18 to 20 hours in media buffered with Tris or
sphite. The rate of growth is reduced when dialyzed serum is employed
the basal medium instead of NHS, but otherwise the results are basically
same. The addition of 0.005 to 0.01 M sodium bicarbonate to the media
taining Tris or phosphite increases the growth rate to that obtained in the
trol (0.02 M bicarbonate) cultures. Under the appropriate conditions,
icient metabolic CO$_2$ is produced to permit maximal growth in Tris or
sphite. For example, cultures with a fluid to air volume of 2, which are
iated with 2 to 3 $\times$ 10^5 cells per ml., grow at comparable rates in bicar-

bonate, Tris, and phosphite. When the rate of growth is restricted by limiti concentrations of bicarbonate, the rate of glucose utilization is likewise retar (FIGURE 2). This probably reflects a reduction in cell number rather tha direct relationship between the concentration of bicarbonate and gluc utilization.

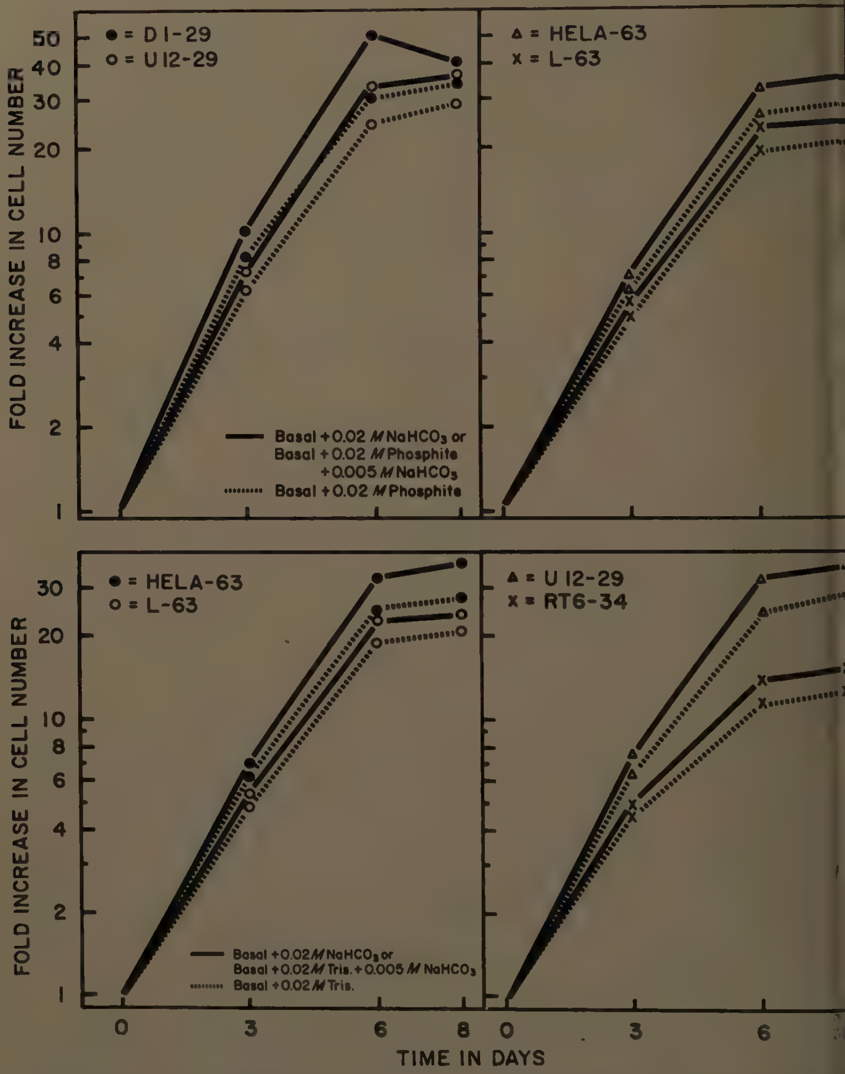

FIGURE 1. Basal medium composed of S77 without bicarbonate fortified with 20 per horse serum.

Changes in pH as a function of buffer system employed are also illustra in FIGURE 2. It should be noted that the pH decreases at a significantly grea rate with phosphite than with Tris or bicarbonate. Both D1S1-48 and N1S

erate *p*Hs as low as 6.5, and it appears that under these conditions the
cose is the growth-limiting factor.

The foregoing data imply that bicarbonate is an essential nutrient for mam-
lian cells *in vitro*. This is demonstrated clearly by the results of experi-
nts summarized in FIGURE 3. In this instance cells were grown in stationary
tures in S77 without bicarbonate supplemented with 5 per cent dialyzed

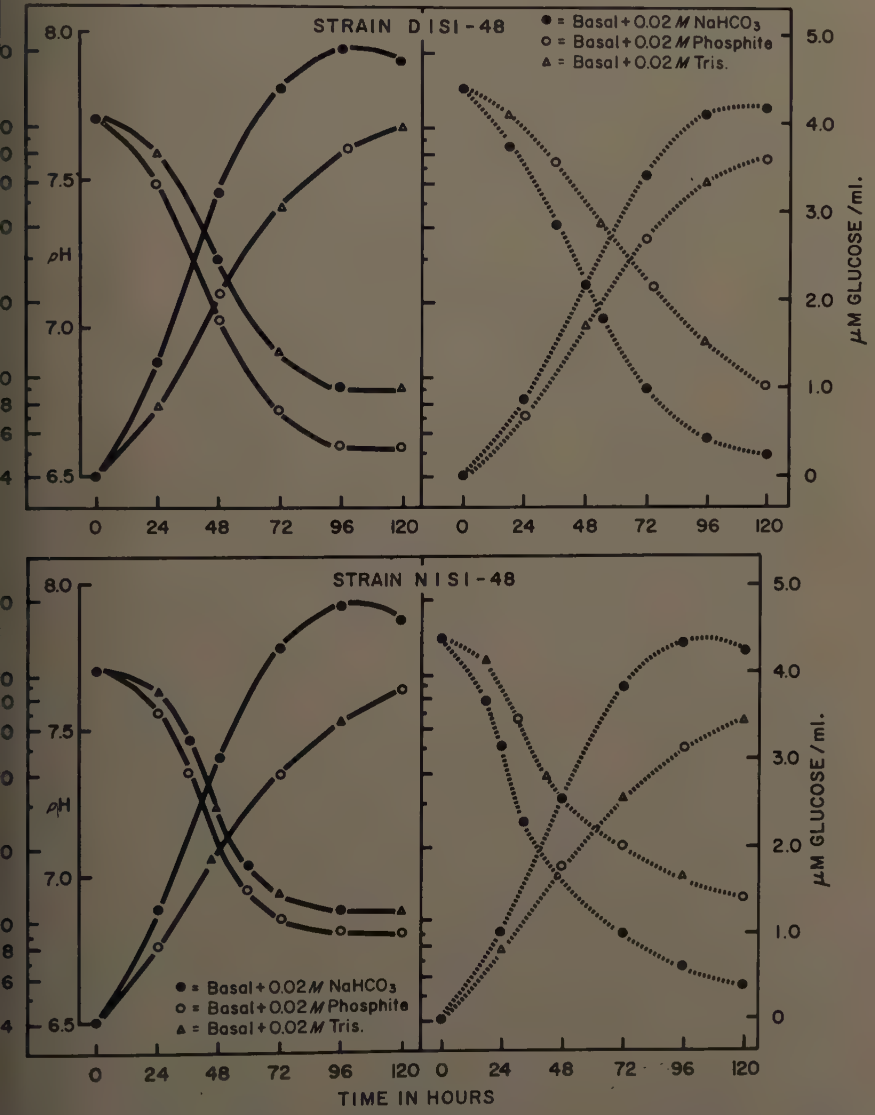

FIGURE 2. Solid line indicates basal medium composed of S77 without bicarbonate
plemented with 20 per cent horse serum and 0.1 per cent Pluronic F68. Broken line in-
ates basal medium composed of S77 without bicarbonate fortified with 20 per cent dialyzed
um and 0.1 per cent Pluronic F68.

horse serum (DHS) and 0.02 M Tris or phosphite, at a final pH of 7.2 to 7 In flasks closed only with loose cotton plugs (open flasks) the cells fail proliferate appreciably and show degenerative changes after 6 to 8 da Longer periods in the absence of added bicarbonate result in death of the cel Under conditions where metabolic carbon dioxide is retained in the syst (closed flasks), the cells proliferate at a rate less than, but comparable to, th observed in the corresponding medium containing 0.02 M bicarbonate.

These observations confirm earlier studies in this laboratory[9] and those Geyer and Chang,[12] which demonstrate that bicarbonate is an essential nutrie for mammalian cells *in vitro*. In this respect mammalian cells do not dif from bacteria and other free-living microorganisms.[13] The importance of C in the nutrition of animal cells in culture is further exemplified by the expe

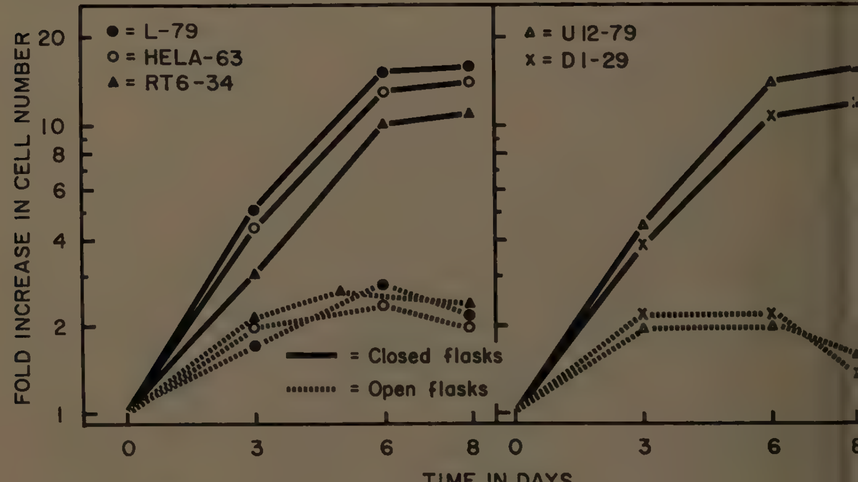

FIGURE 3. Basal medium composed of S77 without bicarbonate supplemented with per cent dialyzed horse serum.

ments of Chang[14] and of Zwartouw *et al.*,[15] which demonstrate that the multip cation of a number of viruses is prevented or restricted in bicarbonate-deplet cells. The ability of bicarbonate-depleted cells to produce virus is restor by the addition of bicarbonate, or ribonucleic acid, or nucleosides and nucle tides obtained from the hydrolysis of ribonucleic acid.[14] On the other har it has not been demonstrated that such compounds support growth under th conditions. Similarly, growth is suboptimal in media containing high lev of embryo extract and serum when metabolic carbon dioxide is permitted escape from the flasks.[9] It appears that carbon dioxide is a multifunctio nutrient, as might be predicted on the basis of the wide array of biochemi reactions in which it participates.[16,17] In the absence of information regardi compounds which substitute for bicarbonate nutritionally, it is evident t the practical application of fixed buffers as a substitute for bicarbonate dependent on the use of a closed system. That is, the use of fixed buff provided they are noninhibitory, appears to be limited only by the ability

cells to produce sufficient metabolic carbon dioxide to satisfy their nutri-
ial needs for this compound. The studies of Gwatkin and Siminovitch[18]
icate that the addition of an internal source of bicarbonate such as oxalace-
might be useful in instances where an insufficient quantity of CO_2 is pro-
ed or retained to provide the nutritional requirements for optimal prolifera-
.

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THE USE OF AMINE BUFFERS IN THE TRANSPORTATION OF FISHES*

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Introduction

The methods of handling live fishes during marking, weighing, and tra port have changed considerably in the last decade. The techniques of h dling and the kinds of modern equipment in use are reviewed by Norris *et* (1960). In general, recent improvements in fish handling have resulted fr better mechanical systems for handling and hauling fishes or from the int duction of chemical agents to the water that quiet the fishes or minim changes in the water resulting from metabolic activities.

One problem that often is correlated with mortality during transport is decline in *p*H of the tranport water resulting from the accumulation of me bolic carbon dioxide. During attempts to stabilize *p*H and control carb dioxide accumulation during fish transport, McFarland and Norris (19 screened several compounds and found that the amine buffer, tris-(hydrox methylaminomethane (hereafter called Tris) was the most suitable. Th results show that Tris: (1) was readily soluble and compatible with both water and fresh water and did not cause precipitation of calcium; (2) provi maximum buffering in a *p*H range from 7.5 to 8.5, a range beneficial for m fishes; (3) did not affect 28 species of fishes in concentrations from 2 to mmoles/l.; and (4) under experimental conditions produced as much a 3-fold extension of the time to mortality of fishes by producing a marked bilization of *p*H, as compared to controls. The disadvantage of Tris for s applications is that the high initial *p*H of the water (above 9) must be redu by the addition of acid. We conclude in this paper that Tris, in spite of necessity of back titration with acid, could be extremely useful in the tra port of fishes in sealed containers where carbon dioxide could not escape, s as the air-shipment of fishes sealed in polyethylene bags. In addition, it suggested that Tris might be useful in controlling *p*H in aerated transp tanks. Often *p*H has been shown to decline sharply from initial values such systems, even though aeration offered escapement of carbon dioxide the atmosphere.

In this paper the buffering capacity of Tris toward carbon dioxide is am fied, and improvements and limitations in its use for fish transport are cussed. In addition, a method that utilizes Tris for measuring the product of respiratory carbon dioxide by aquatic organisms is described.

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A Comparison of the Hydrogen Ion and Carbon Dioxide Buffering Capacity of Tris

McFarland and Norris (1958) indicate that Tris at concentrations of 11 mole/l. and 44 mmole/l. is capable of increasing the hydrogen ion absorpon capacity of sea water between *p*H 7.5 and 8.5 by 15 and 50 times, respecvely. However, results were based on acid titrations of buffered solutions at were not corrected for dilution. A series of acid titrations of increasing ncentrations of Tris in coastal sea water, corrected for dilution (FIGURE 1), ow that the buffering capacities are lower than previously reported. For tance, hydrogen ion (H^+) absorption capacities, calculated from the data FIGURE 1, give increases compared to nonbuffered sea water of 5.6, 12, 24.8, and 48.8 for concentrations of 4.4, 11, 22, 44, and 110 mmole/l. of sea ter, between *p*Hs of 8.5 and 7.5

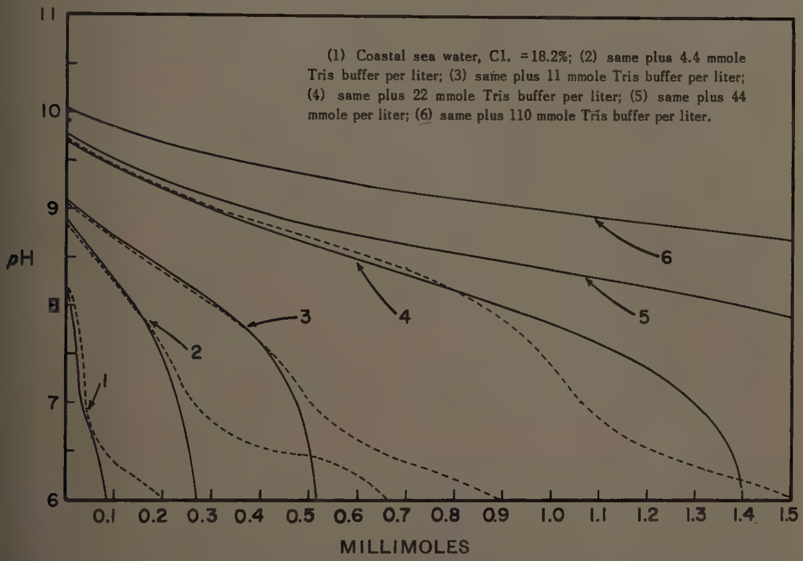


FIGURE 1. Hydrochloric acid and carbon dioxide titration curves for Tris-buffered and buffered sea water. The abscissa represents millimoles of hydrogen ion or of carbon dide per 50-cc. sample. Curves 1 through 6 represent results for the concentrations of Tris fer indicated in the legend of the figure. Solid lines are curves for the titration of samples h 0.10 *N* HCl. Dashed lines are curves for titration of samples with carbon dioxide satted water. Analysis of water saturated with carbon dioxide, used as the titrant, with a kman Infrared Analyzer showed carbon dioxide to be 6 per cent supersaturated. All ves are corrected for the effect of dilution during titration. A comparison of buffering acities is given in the text between *p*H 7.5 and 8.5.

Recently a mixture of Tris and its hydrochloride has been made available der the name of Trizma-8.3.* It yields a *p*H varying from 8.4 at 20° C. 8.13 at 30° C. regardless of concentration. Acid titration curves for Trizma various concentrations are plotted in FIGURE 2. They show that buffer

* Sigma Chemical Co., St. Louis, Mo.

capacities in sea water between pH 8.5 and 7.5 agree with capacities obtai for Tris. Trizma has the important advantage of eliminating back titrat prior to introduction of fishes in transport operations. It has been used clusively in this study other than for the data reported in FIGURE 1.

McFarland and Norris (1958) demonstrated that Tris at a concentration 22 mmole/l. extended the time to 50 per cent mortality of fishes in sealed c tainers from 2.25 to 6 days. This extension of time to mortality seems short of the potential H^+ buffering capacity of the buffered sea water

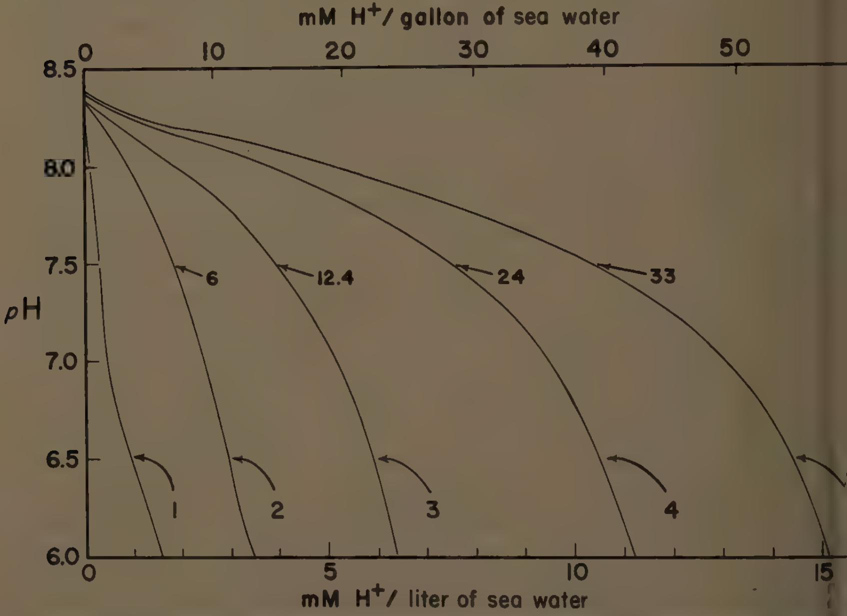


FIGURE 2. Hydrochloric acid titration curves for increasing concentrations of the hy chloride derivative of Tris (Trizma-8.3) in coastal sea water. Curve 1 represents nonbuff sea water, while curves 2 through 5 contain Trizma-8.3 at concentrations of: 2 gm./ga gm./gal., 10 gm./gal., and 20 gm./gal. respectively. Numbers indicated by arrows for cu 2 through 5 at pH 7.5 represent the increased buffer capacity toward hydrogen ion comp to curve 1, down to a pH of 7.5.

mmole/l. = 24-fold increase), but careful examination of these earlier d reveals that H^+ absorption capacity to pH 7.5 was 26 times the contr This capacity agrees closely with the values of 24.8 and 24 reported here similar concentrations of Tris and Trizma buffer (FIGURES 1 and 2). Si the acidifying agent produced by fishes is not H^+ but carbon dioxide (C the data indicate that metabolic CO_2 above pH 7.5 is acting toward the bu as if it were a strong acid.

If metabolic CO_2 acts as a strong acid toward Tris, simple acid titratio buffered solutions should allow calculation of the amount of CO_2 that car absorbed. A method of titration reported by Beyers and Odum (1959) t utilizes CO_2 saturated distilled water as titrant, was used to determine if reacts toward Tris in the same way as H^+. Results of these titrations

ted in FIGURE 1 as dashed lines. The data clearly show that at low con-
trations of Tris (below 11 mmole/l., curves 2 and 3) equimolar concentra-
ns of CO_2 and H^+ have the same effect in lowering the *p*H of buffered solu-
ns. This effect holds above a *p*H of 7.5, whereas below this *p*H the CO_2
ation curve tends to flatten. The flattening is attributible to the buffer-
action provided by the $HCO_3^- \rightleftharpoons H_2CO_3$ equilibrium. Similar *p*H curves
e been found for fishes respiring in sealed containers (McFarland and
rris, 1958).

t higher concentrations of Tris, equimolar *p*H effects for CO_2 and H^+ exist,
once again deviations occur as the *p*H declines. For instance, in FIGURE
urve 4, below a *p*H of 8.3 the effect of CO_2 on *p*H change is greater than
Even at higher concentrations of buffer the flattening near *p*H 7.5 oc-
s. Repeated titrations always gave this result. At higher concentrations
Tris (FIGURE 1, curves 5 and 6) a deviation to the left also occurred, but
ilts are not plotted since high dilutions with the CO_2 titrant solution were
iired to lower the *p*H below 8, and probably this tended to amplify titra-
errors. Therefore it must be concluded that as Tris concentration in-
ises, acid titrations tend to produce overestimates of the CO_2 buffering
acities of the solutions. For instance the calculated 24.8-fold increase in
acity for 22 mmole/l. tris buffered sea water, when compared to nonbuff-
l water between *p*H 8.5 and 7.5, is only 13.3 when measured with CO_2
ead of H^+. Under these conditions 6.6 mmole CO_2/l. of buffer was as
ctive as 11 mmole H^+/l. of buffer. These deviations with increased con-
tration remain unexplained.

xamination of curves 1 in FIGURE 1 for nonbuffered sea water indicate that
is not as effective as H^+ in reducing *p*H. Repeated titrations substan-
ed this tendency. From 20 to 30 per cent more CO_2 was required than H^+,
a molar basis, to produce a given *p*H reduction. Similar results for CO_2
sus H^+ titrations with natural waters have been reported by Beyers and
m (1959). Beyers and Odum attribute this deviation from equimolarity
common "ion" effect since CO_2, the titrant, is added to a system that
ady contains the carbonate system. Verduin, in an exchange of published
ers (Verduin *et al.*, 1960), disagrees with this interpretation and suggests
H^+ and CO_2, mole for mole, should produce similar changes at naturally
irring *p*H's (7.5 to 9). It is interesting that the titration curves for low
centrations of Tris support Verduin's conclusion, while the nonbuffered
ves support Beyers and Odum's contentions. It may be that the buffered
tions completely bind CO_2 so that a common "ion" effect in the solution
not occur and therefore CO_2 and H^+ produce equimolar *p*H declines. A
l solution to this most interesting controversy must await CO_2 titration
edures that assure no escapement of CO_2 from the nonbuffered solutions
ng titration. In our titrations of nonbuffered water *p*H was often noted
ise slightly even when titrations were performed rapidly, a result we attri-
to CO_2 escapement.

Beyers and Odum's conclusions are correct, the buffer capacity of Tris
O_2 might be lower than indicated for the data of FIGURES 1 and 2. Assum-
that from 20 to 30 per cent more CO_2 is required than H^+ for nonbuffered

sea water, the actual buffering capacities of Tris solutions for fish will be 75
85 per cent of the stated values. Even considering these reduced values t
CO_2 absorption capacity of Tris is considerable and most useful in closed-sy
tem fish transport. However, data presented later indicate that Tris, with
its buffering range, completely absorbs CO_2 and therefore reductions in buff
capacity probably do not occur.

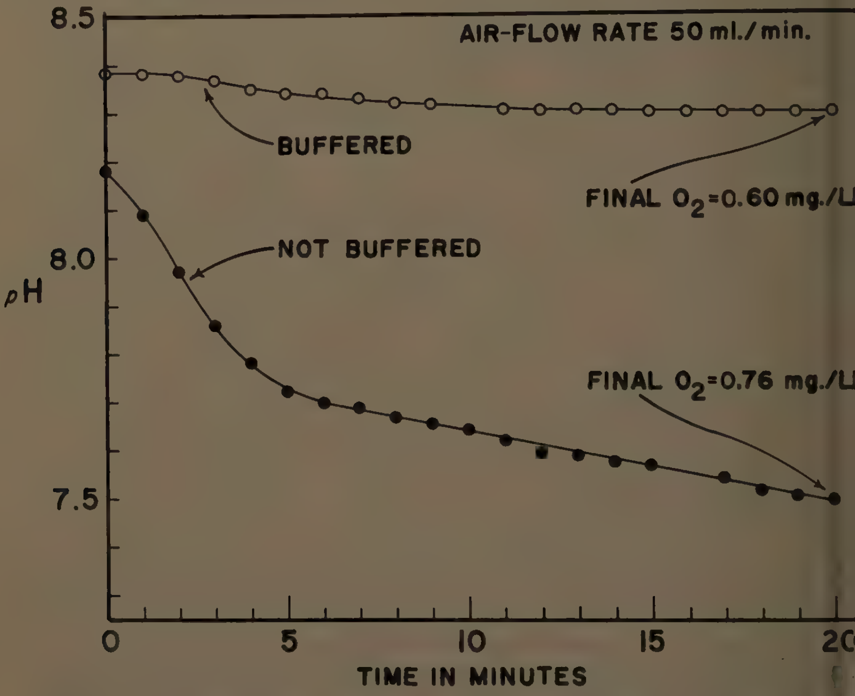
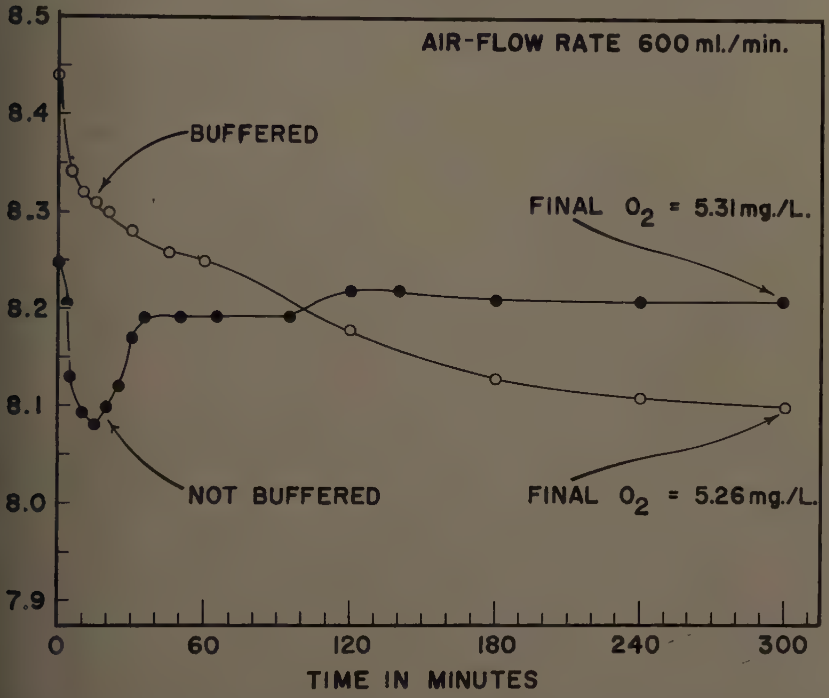


FIGURE 3. The effect of low air flow rate and of Trizma-8.3 buffer on pH decline in a t
containing 1 lb. of the pupfishes *Cyprinodon variegatus* per gallon of sea water. Air flow r
represents cc. of air/min./l. of water. Concentration of buffer was 10 gm./gal. or, appro
mately, 22 mmole/l.

The Use of Tris for Open-system Transport

In our earlier work (McFarland and Norris, 1958) we suggested that T
might be useful in fish transport systems that are not sealed and that commo
employ aerating devices. Preliminary investigation, during actual transp
seemed hopeful. A group of marine tropical fishes held in open-system tra
port tanks for a 12½-hour period between Los Angeles and San Francis
Calif., underwent a pH decline in nonbuffered sea water from an init
value of 7.95 to a final value of 7.4. A Tris-buffered tank changed only fr
8.25 to 8.20 during the same run. Both the weights of fishes carried per u
volume of water and the rates of aeration were considerably lower than
countered in most fish transport operations. Average loads of trout tra
ported by most fish and game agencies are close to 1 lb. of fish per gallon
water (Norris *et al.*, 1960).

o clarify further the usefulness of Tris for open-system fish transport tanks eries of experiments were performed with 1 lb. of fish per gallon of water with variable rates of aeration. The pup fish *Cyprinodon variegatus* was d as the experimental animal. In these experiments 120 gm. of *Cyprinodon* proximately 200 fish) were weighed and placed in 1 l. of sea water held in cylindrical battery jars. A *p*H electrode was inserted and *p*H decline re- led against time in Tris-buffered and nonbuffered water for variable rates



IGURE 4. The effect of high air-flow rate and of Trizma-8.3 buffer on *p*H decline in a containing 1 lb. of the pupfishes *Cyprinodon variegatus* per gallon of sea water. Air-flow represents cubic centimeters of air/min./l. of water. Trizma-8.3 concentration was 10 gal. Note that the *p*H of the nonbuffered group attains equilibrium, while the *p*H of buffered group declines. Oxygen concentration of the water with a high air-flow rate, e results in FIGURE 3, is balanced against the oxygen requirement of the fishes and re- s high.

ir flow. Results from these experiments for a low- and a high-aeration are shown in FIGURES 3 and 4. Air flow rates were determined by collect- the gas emitting from the aerating stone after initial equilibrium was at- ed. Following this measurement the groups of fish were introduced.

ypical declines in *p*H were obtained for the nonbuffered groups of fish re aeration rates were low (FIGURE 3). The *p*H declined to 7.5 by 20 min. oxygen content of the water also showed a drastic reduction. All fish were er severe stress at 20 min. Under similar conditions, but with 22 mmoles ma/l., the *p*H was relatively stable over the 20 min. period (FIGURE 3). ever, this group of fish also displayed stress typified by swimming at the surface and loss of equilibrium. In this group, and in others like it, the fi oxygen content of the water was always somewhat lower than in nonbu ered groups. McFarland and Norris (1958) suggested that one advantage of bu ering during transport was that pH stability would offset the marked B effect that occurs in fish bloods when CO_2 increases. It is felt that the cons ently lower oxygen for the buffered groups is the result of better oxygen ut zation since a Bohr effect is eliminated. However the data clearly indic that buffering, where oxygen demand is high and supply is low, has no vantage in transporting fish, other than pH stabilization.

In the situation where the aeration rates are sufficient to balance the oxyg requirements of the fishes, the disadvantage of Tris in open-system transp is clearly shown (FIGURE 4). Nonbuffered groups show an initial sharp d in pH, followed by a rise in pH until equilibrium is attained slightly below initial pH. Oxygen is maintained at a high level and indicates the bala between oxygen demand and supply. In buffered groups the pH decli steadily. This is attributible to the high affinity of Tris solutions toward (see next section and previous section on CO_2 titrations). The balance oxygen is indicated by the similar high values obtained for both the buffe and nonbuffered groups at 5 hours when the experiment was terminated. experiments with sufficient aeration neither buffered groups nor nonbuffe groups showed signs of stress.

The data indicate that high aeration rates where oxygen demand is high more effective in maintaining high pH than buffering. Apparently scaveng of CO_2 to the atmosphere, a process combined with oxygenation of the wa can be most effective. It is concluded from the CO_2 titration results for T and the data presented in the next section that CO_2 is tightly bound, whet directly or indirectly, at least down to a pH of 7.5. At this pH, a level consider minimal for transport of marine fishes, decreased oxygen affinity of blood through a slight Bohr effect can occur and can conceivably result in insufficient supply of oxygen to the tissues. If our experiments had not b terminated at 5 hours but continued, pH of the buffered solution would h reached a pH of 7.5. The high aeration rate would not be expected to count act the pH decline nor the acidic properties of the saturated buffer. Therefo an insufficient supply of oxygen to the tissues could ensue and particularly the pH declined below 7.5. The necessity of maintaining oxygen supply to tissues during fish transport has been indicated by Norris *et al.* (1960) means of combating this stress are more fully discussed by McFarland (19

Although the results with Tris in open-system fish tanks indicate that i of limited use the entire question needs further examination. For instance fish loads were heavier (up to 3 lb./gal.) addition of Tris to the water mi be an advantage if aeration would not equilibrate the pH decline in nonbuffe water resulting from the increased CO_2 production. Until further work on interactions of varying weights of fish per unit volume, variable aeration ra variable ratios of surface area to water volume of tanks, and longer time peri is done, and their effect on decline of both pH and oxygen determined, ultimate answer to the usefulness of Tris in open-system tanks cannot be giv

It is apparent, however, that the main advantage of using Tris during transport is not in open-system tanks but rather in closed systems where not escape. It should therefore find its major utility in the air-shipment ish. Where aeration is sufficient to meet oxygen demand and loads of fish light (less than one eighth lb./gal.), Tris can be utilized to maintain higher if desired, but its over-all utility in open-system transport seems negative.

The Measurement of Carbon Dioxide Production of Fish Using Tris

'he measurement of the CO_2 production of aquatic organisms is a most icult task since the CO_2 from metabolism enters into equilibrium with the ieous phase. Carbon dioxide is often calculated from *p*H change, but this ffected by a series of variables such as alkalinity and temperature. Accuy requires a thorough knowledge of these parameters and their interactions h CO_2 to change *p*H. In addition, unless experiments are performed in sed vessels, CO_2 escapement can cause serious underestimation of CO_2 production.

method that might simplify analysis of CO_2 production using Tris was idently discovered during a series of separate investigations in which it desired to determine the diffusion rates of inert gases into the teleost m bladder. The apparatus used was a large respirometer consisting of a . ground glass cylindrical battery jar fitted with a three-fourths-inch airit Lucite cover. During experiments 15 l. of sea water were added leaving l. airspace. Projecting through holes in the cover were a manometer, a -phase outlet extending approximately 2 inches into the airspace, an inlet ending into the water and fitted with an aerating stone, a stopcock connected tank of medical oxygen, and a syringe needle stopper for both air-phase and eous-phase sampling. A Cole-Parmer air pump was connected to the outlet inlet so the air-phase gases could be pumped out and circulated back into aqueous phase through the air stone. This provided rapid mixing and ilibrium of gases throughout the respirometer. The entire apparatus was merged in a large aquarium for temperature control with the exception of pump and manometer.

'he aqueous phase was monitored for *p*H and the gas phase analyzed for with a Beckman Model GC-1 gas chromatograph using a silica-gel column. pinfish *Lagodon rhomboides* was used in all experiments. Oxygen consumps of fish were determined by displacement of the water column in the iometer. Manometer calibration was achieved by previous injection of wn amounts of gas. Medical oxygen was used to replace consumed gen.

esults from one such experiment are plotted in FIGURE 5. In the nonbuff- control experiment the fish weighed 54.5 gm. and the fish in the buffered eriment weighed 65.5 gm. Oxygen consumption for these fish over the ¼-hour experimental period varied from 0.32 to 0.34 cc. O_2/gm. body ght/hour for the control and from 0.30 to 0.33 cc. O_2/gm. body weight/hour he fish in Tris. That Tris had no effect on oxygen consumption of fish is cated by the agreement with controls. Replicate experiments with other ish gave essentially identical results. McFarland (1960) found no differ- between oxygen consumption of buffered and nonbuffered groups of the eye *Girella nigricans*.

The *p*H curves of FIGURE 5 follow the expected pattern, the nonbuffer water showing a rapid drop that flattens out below *p*H 7.5, while the buffe water shows a slower decline that is almost linear. Eleven mmoles Trizma was used to buffer the water, and separate samples of this solution as well the nonbuffered sea water, were titrated with CO_2 to determine change cubic centimeter of CO_2 gas.

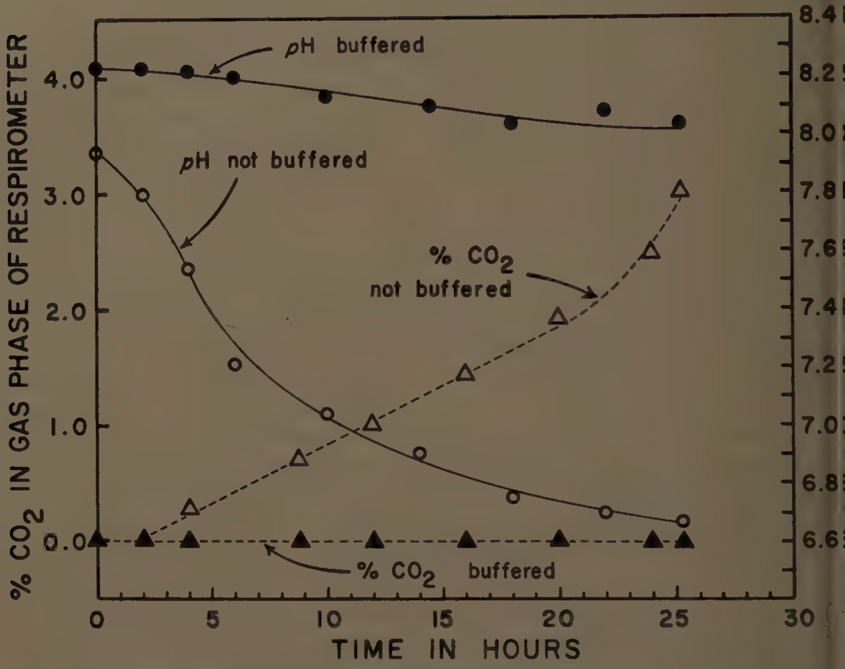


FIGURE 5. The effect of carbon dioxide respiration by the pinfish *Lagodon rhomboide* *p*H decline and carbon dioxide accumulation in a respirometer for Trizma-8.3 buffered nonbuffered sea water. Note that carbon dioxide does not accumulate in the air phase of respirometer in buffered water, but accumulates linearly in the air phase of the nonbuff water at a *p*H of 7.5 and lower. Weights of individual fish were 65.5 gm. for the buff water and 54.5 gm. for the nonbuffered experiment. Oxygen and carbon dioxide rates 0.32 cc. O_2 and 0.27 cc. CO_2 per gm. of body weight per hour for the buffered experim and 0.33 cc. O_2 and 0.21 cc. CO_2 per gm. body weight per hour for nonbuffered experim (see text for discussion).

From this data the CO_2 production of the pinfish could be determined change in *p*H. During a 25¼-hour period in the buffered respirometer a weighing 65.5 gm. respired an average of 0.27 cc. CO_2/gm. body weight/h From this rate we calculate an R.Q. of 0.84 (0.27/0.32). In contrast, nonbuffered fish respired an average of 0.21 cc. CO_2/gm. body weight/h resulting in an R.Q. of 0.64 (0.21/0.33). In other experiments R.Q.s of 0 0.88, and 1.01 were obtained for pinfish in Trizma and values of 0.66 and obtained for pinfish in nonbuffered water. The R.Q.s obtained for fish Trizma buffer, with the exception of the one high value are in the expe range for fishes, whereas the values for the nonbuffered experiments are sistently low. This may be explained partly by escapement of CO_2 to the phase of the respirometer (FIGURE 5). However, if we assume that buffered l nonbuffered R.Q.s are actually the same, calculation of the volume of CO_2 the air phase of nonbuffered tests shows that approximately 30 per cent of gas is missing. This discrepancy is best attributed to diffusion of CO_2 to atmosphere through the tubing that connected the air circulating pump the respirometer.

The gas analysis of the air phase for the buffered experiments shows that e CO_2 at all times was undetectable. Trizma, therefore, completely trapped the metabolic CO_2. In the nonbuffered experiments, free CO_2 was first itted into the air phase between pH 7.8 and 7.5 (FIGURE 5). Interestingly s CO_2 escapement was always almost linear, suggesting that the respiration he fish was essentially linear through the experiments.

Technical improvements in apparatus most certainly could improve the pre- on of these respiratory measurements. However even these relatively de results are encouraging, and they suggest a method whereby heretofore icult problems in respiratory physiology might be undertaken. For in- nce, since Tris effectively traps metabolic CO_2 it might be used to measure $_2$ production in large aquatic organisms such as sharks and tunas held in n tanks. Previous determinations of the effect of atmospheric CO_2 on ks containing Tris as a function of time could be subtracted from the joint ion of atmospheric CO_2 and metabolic CO_2 on pH of the water. The CO_2 duction of these large animals could be established using data on the rela- n between CO_2 and pH for the particular water, determined empirically by $_2$ titration. Proper choice of buffer concentration for a particular rate of piration should allow a sufficient change in pH with time for accurate pH erminations.

Summary

1) The buffer capacity of the amine buffer, tris(hydroxy)methylamino- thane (Tris), toward both hydrogen ion and free carbon dioxide is deter- ned, between pH 7.5 and 8.5. Concentrations of 4.4, 11, 22, 44, and 110 nole Tris/l. increase the H^+ capacity by 5.6, 12, 24.8, 34, and 48.8 over buffered coastal sea water. Trizma-8.3, a hydrochloride salt mixture of s, gives similar H^+ capacities. Carbon dioxide capacities of Tris are equal, le for mole, to H^+ capacities at concentrations of Tris of 11 mmole/l. or er down to a pH of 7.5. At pHs below 7.5, titration curves for CO_2 show attening similar to that produced by fish respiration. At higher concen- ions deviations from equi-molarity of CO_2 and H^+ occur.

2) Limitations in the use of Tris for fish transportation are discussed, and s concluded that Tris is most valuable where fish are transported in sealed tainers. In open systems aeration is more effective in maintaining high of the water than buffering, since the buffer traps CO_2 and, in so doing, uces pH.

3) A method utilizing Tris for the determination of CO_2 production by atic organisms is developed, based on the slow and linear decline of pH Tris-buffered water and the empirical determination of the effect of CO_2 on

*p*H of water. It is suggested that modification of this method might adapted to the measurement of respiration of large aquatic organisms, such sharks and tunas.

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MECHANISM OF THE SELECTIVE FLUX OF SALTS AND IONS THROUGH NONAQUEOUS LIQUID MEMBRANES*

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Columbia University, New York, N. Y.

Iost living cells possess asymmetric membranes in which the concentration ions in the interior is quite different from that in the extracellular fluid. e unequal distribution of ions plays a major role in the generation of small ctric currents that conduct the nerve impulse. This condition appears ociated with an exchange of extracellular sodium with intracellular potasm. J. H. Schulman and H. L. Rosano[1] have used a liquid membrane model studying the selective flux of various salts through short chain alkyl alcohols. e apparatus consisted essentially of a thermostated polystyrene box with artition. An aqueous solution was placed in each compartment over which s floated a nonaqueous liquid membrane (FIGURE 1). The advantage of a uid membrane is twofold: interfacial orientation; interfacial homogeneity sus pore size with a solid membrane. The use of a physicochemical model kes it possible to investigate an initially simple system in which the comxity of the actual membrane can be achieved by adding different physiocal components.

chulman and Rosano have studied the diffusion of different salts through erent oil layers under varying conditions. This phenomenon was called usion transport. In the presence of an amphoteric phospholipid (carrier) he oil membrane interface, the transport is markedly increased; this phenomn was called carrier transport. This carrier transport functions also when diffusion process has been blocked.

his article is primarily an extension of this work. With regard to the ct of biological components upon the selective flux of salts and ions through aqueous membranes, a review of the diffusion transport will be given.

Diffusion Transport

V is the volume of oil and A the area of the two identical oil-water inters, the cell is equivalent to a membrane of V/A thickness; this is true pro-ed that the thickness of the oil layers is sufficient to allow for a fairly homoeous system when stirred. The volume of the oil phase is directly related he partition coefficient of the oil-water system and therefore is related to diffusion transport. By equating the diffusion transport phenomenon to a usion across an homogeneous membrane in the same mathematical form as general flux equation for electrolytes,[2] the results may be represented by uming the following equation:

$$\frac{dC_2}{dt} = \frac{KAP_{0\omega}}{2V}[C_1 - C_2] \tag{1}$$

The work described in this paper was supported by Research Grant B-2067(C) from the onal Institute of Neurological Diseases and Blindness, Public Health Service, Bethesda,

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where $P_{0\omega}$ is the partition coefficient; C_1, the salt concentration of one aqueo phase; C_2, the salt concentration in the opposite aqueous phase; and V, volur of the aqueous phase. A is the area of one of the identical oil/water interfac K is a coefficient. In our case, the partition coefficient was substituted for t electrical potential function, since the salt concentration in the oil layer is

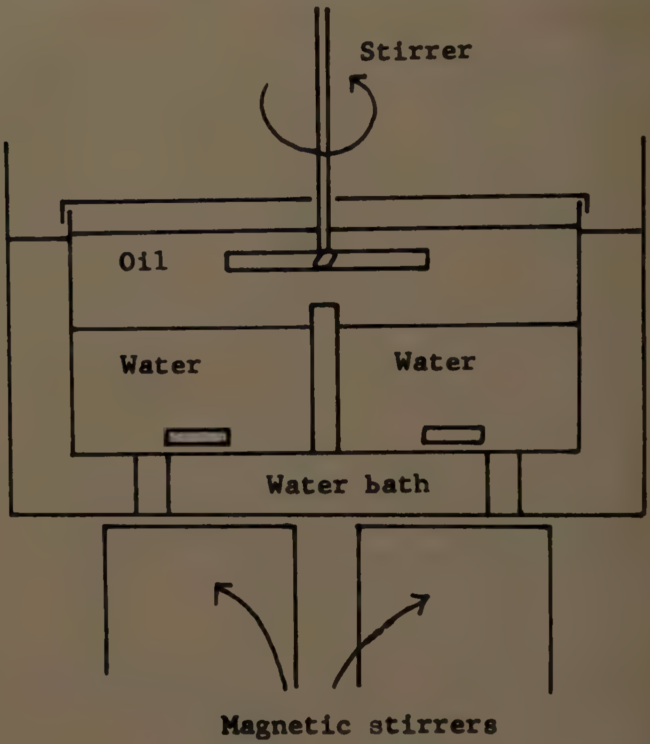


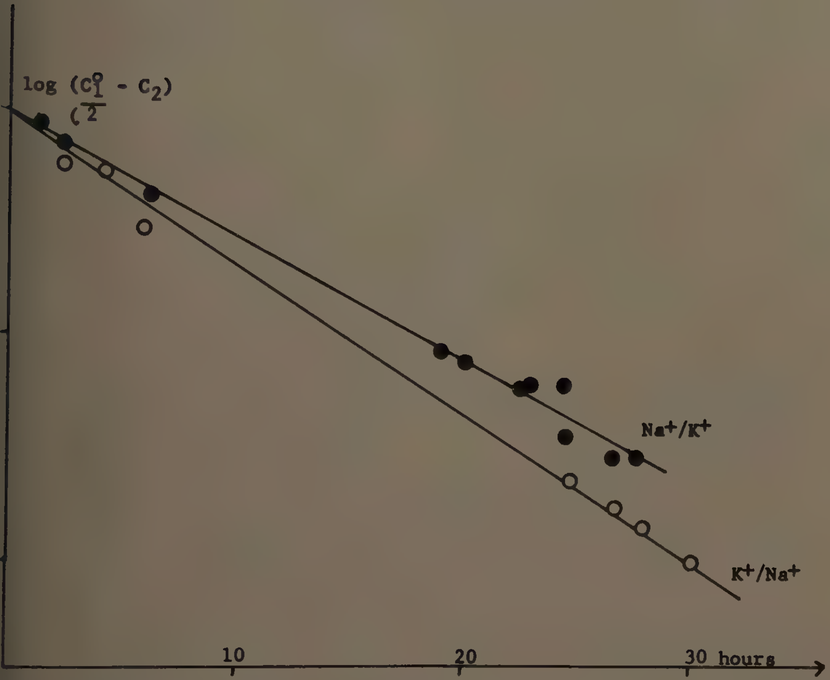
FIGURE 1. Transport cell.

ways small. Consequently, $C_1 + C_2 = \text{constant} = C_1^0$ (initial salt concent tion). Then by substituting in **1** and integrating, this gives (t = time)

$$\log\left[\frac{C_1^0}{2} - C_2\right] = -\frac{2KAP_{0\omega}t}{V}$$

To a first approximation the results fit this equation. In the case of diffusi transport through various oil membranes, the partition coefficient was t primary controlling factor. Secondary effects neglected in this discussi such as energy barrier at the interface, electrical effects, stereochemistry, a ionic hydration, will be discussed in a subsequent paper.[3] FIGURE 2 illustra our results.

In the case of diffusion transport, the $P_{0\omega}$ plays a major role; the directi of the salt flux appears to have no effect. However, in the carrier transp em this is not the case, since the direction of the salt flux does have a con-ling effect, as does the degree of adsorption of the carrier at the interface. the previous publication,[1] where diffusion occurs, isotonic conditions were ntained in order to prevent water transport, since the system acts as an ometer when there is no isotonicity. The rate of water transport by the otic effect appears to be primarily related to the water content of the rated oil phase, as well as the difference in salt concentration in the 2 eous compartments. In the case of 1-pentanol saturated with water (3.5



GURE 2. Linear representation of the diffusion of 50 mmole/l. of NaCl and 50 mmole/l. Cl through 1-butanol at 30° C.

ent by volume at 30° C.) and differences of 50 mmole in salt concentration, change in the aqueous volume was found to be negligible. This is not for alcohols containing more water, that is 1-butanol 6.9 per cent; 2-nol 27.5 per cent; 2-methyl 2-butanol 12.3 per cent per volume at 30° C. JRE 3), where saturated NaCl was placed in one compartment and the me increase was measured with time.

was found that the electrical potential measured across the nonaqueous brane varies linearly with the difference in salt concentration. Two nel electrodes in KCl solution were connected to the aqueous compart- with 2 capillary siphons. The whole was thermostated at 30° C. with a r bath. No electrical potential was measured when the oil layer was re-d by a KCl bridge. Ten mmole/l. of NaCl were opposed to 100, 250, 500, 1000 mmole/l. of NaCl and identical experiments were performed with K (FIGURE 4). The potentials were measured with a Keithley electrome model 610A, accuracy 1 mv. The potentials measured with this system w appreciably lower than those expected from the Nernst equation, which p dicted a change of 58 mv for a tenfold change in salt concentration. Fr FIGURE 4 we calculated a change of 13.8 mv for a tenfold change of NaCl c centration and 11.85 mv for KCl. Since the Nernst theory implies a me brane impermeable to one of the ions we concluded that the membrane

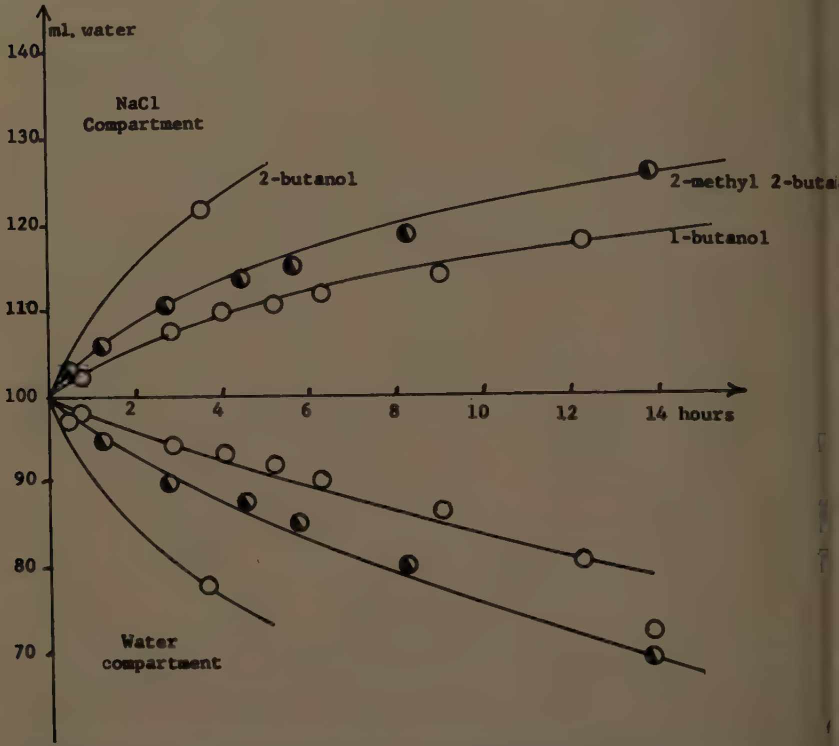
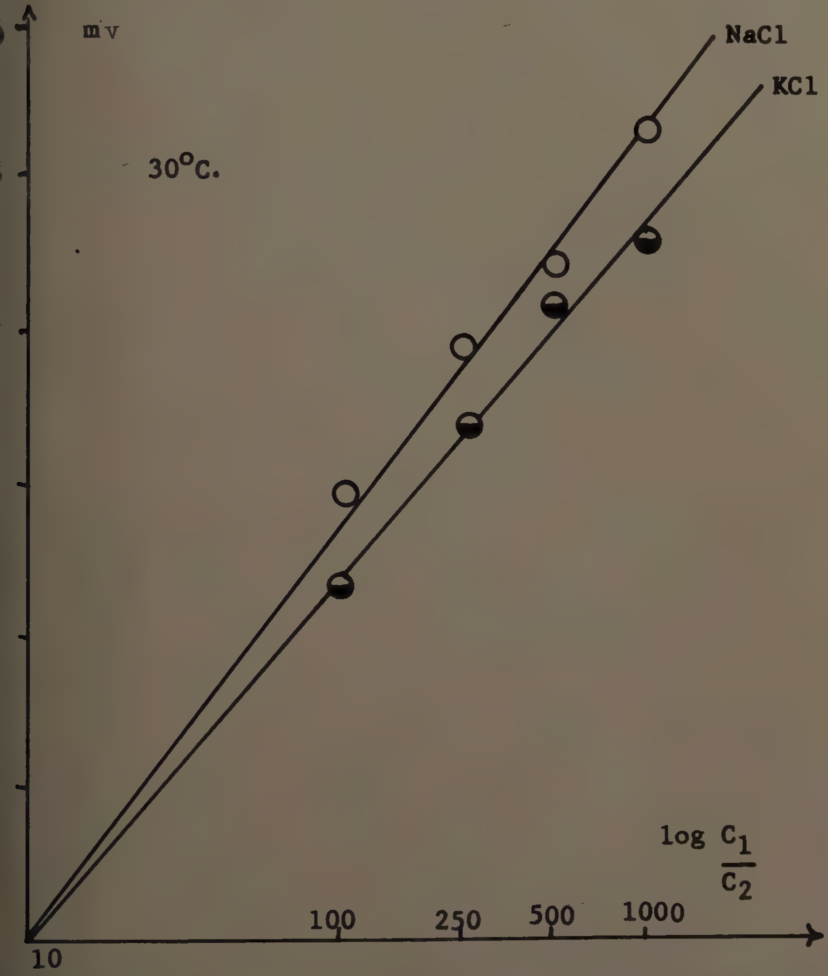


FIGURE 3. Increase and decrease of opposing volumes of 100 ml. of NaCl satur solution to 100 ml. of water separated by various alcohols as a function of time.

permeable to the anions and the cations but that their mobilities are differ This is in agreement with the work of Dupeyrat,[4] who performed similar exp ments with nitrobenzene. The Nernst equation has been modified by Hen son by considering the ionic mobilities in the membrane

$$E = \frac{U - V}{U + V} \frac{RT}{F} \ln \frac{C_1}{C_2}$$

U and V, ionic mobilities; C_1 and C_2, salt concentrations; R, gas constant absolute temperature; F, the Faraday. By using this equation and assur a unit velocity for the chlorine ion, the relative dynamic mobilities for Na^+ and K^+ ions were calculated (TABLE 1). Since the oil phase is homogen results found refer to the rate of movement of the cations through the rface. In the diffusion through the oil membranes it was always found that assium diffuses quicker than Na. It is stated above that the rate of diffu- was primarily controlled by the partition coefficient. In the case of NaCl



IGURE 4. Electrical potential variation across 1-butanol (at 30° C) for identical salt rious opposing concentrations.

TABLE 1

	Ionic mobilities at infinite dilution in water	Relative dynamic mobilities calculated from the Henderson equation assuming Cl^- mobility equal unity
Cl	76.35	1.0
K(KCl)	73.5	.66
Na(NaCl)	50.1	.57

and KCl at 50 mmole/l. concentration at 30° C. the partition coefficients equal at $1.8.10^{-2}$, therefore the difference in ionic mobility explains the diff ence between Na^+ and K^+ fluxes. In water the mobility of the potassium is almost equal to that of the chlorine ion although the sodium ionic mobil is markedly smaller. The mobilities of 2 ions through the 1-butanol membra is less than that of chlorine. The sign of the potential indicates that chlori ions move faster. The diameter of the hydrated ion in water is 3 Å for pota sium and 4.5 Å for sodium.[5] The size of the hydrated ion may be one reas why the potassium ion passes the interface faster than the sodium ion. Oth factors may be the stereochemistry of the alcohol group, its orientation at t interface, and its relation to the hydrated ion. In the case of 1-butanol, t molecule is oriented at the interface with the alkyl chain in the oil phase, a the —OH group in the water phase. The same phenomenon occurs with pentanol. In the case of 1-pentanol, the rate of diffusion of water into t alcohol phase is much lower than with 1-butanol. This is analogous to wa evaporation retardation with long chain alcohols. This decreases exp nentially with the number of carbon atoms in the chain,[6] and no retardation shown with branch chain alcohols (FIGURE 3).

TABLE 2
PARTITION COEFFICIENTS OF KCL IN 1-BUTANOL AT 30° C.

Systems	Partition coefficients
50 mM/l. KCl	1.8×10^{-2}
50 mM/l. KCl + 50 mM/l. NaCl	1.8×10^{-2}
50 mM/l. KCl + 50 mM/l. HCl	0.99×10^{-2}
50 mM/l. KCl + 50 mM/l. NaOH	1.85×10^{-2}

In the case of a branch chain alcohol, only a study of the interfacial surfa isotherm could give an idea of their surface orientation and, consequently, possible mechanism of ion migration through the interface.

It has been shown[1] that the rates of diffusion of NaCl and KCl can be celerated or decelerated by addition of $—OH^-$ or H^+ ions respectively. Th was previously assumed to be due to the difference in their ionic mobilities the oil phase. On the other hand, it has now been shown that the rate diffusion is also dependent on the partition coefficient of the salt between t aqueous and nonaqueous phases. This factor might also be affected by t presence of $—OH^-$ or H^+ ions. This has been demonstrated in TABLE 2, whi shows that the partition coefficient is markedly reduced by the presence of ions and slightly increased by $—OH^-$ ions. The rate of diffusion of NaCl a KCl at 50 mmole/l. level, at which concentration most of the experiments w carried out, were found similar, whether the salts were in the same or t opposite compartment. This is explained by the identical values of the par tion coefficient for the two salts (TABLE 2).

Carrier Transport

It has been shown[1] that the ionic transport in either permeable (1-butan impermeable (1-pentanol), or diffusion-blocked (1-butanol and CCl_4, or tanol and petroleum ether mixture) can be enhanced by the addition of an photeric lipid in the oil phase (cephalin or lecithin at the appropriate *p*H). the case of carrier transport the cation and the anion can exchange inde- dently only when in counterflow. No exchange can take place when the ts are together in one compartment, and opposed to water alone in the other npartment. The difference in mechanism between diffusion and carrier nsport is that the partition coefficient between the oil and the water phase ys a minor role compared to the interfacial partition coefficient between the erphase and oil membrane concentration of carrier molecules. There are o major factors involved: (1) the availability of the carrier molecule at the erface, which is related directly to the dynamic adsorption equilibrium of lipid at the oil/water interface; and (2) the availability of an exchangeable

The two points mentioned can be illustrated by studying the ionic exchange ween NaCl and KCl through a 0.01 molecular weight (M/100) cephalin in roleum ether containing increasing amounts of 1-pentanol. It was found t 30 per cent addition of 1-pentanol to the cephalin-petroleum ether solu- ns brings the Na-K exchange back to that found with 1-pentanol alone as the phase; no exchange takes place with cephalin in petroleum ether alone. is suggests that the 1-pentanol-cephalin association draws the cephalin lecule to the oil/water interface making them available for ionic exchange GURE 5).

The exchange phenomenon of Na^+ and K^+ was also studied through a halin 1-pentanol membrane with increasing concentrations of cephalin. ese results are shown in FIGURE 6. It may be seen on this figure that below oncentration of cephalin of M/400 the carrier transport appears to increase early up to M/100. The concentration of M/100 appears to be close to the centration of saturation of 1-pentanol by cephalin.

I. Schiff (unpublished data) determined the cephalin concentration in a urated 1-pentanol solution by using the Kjeldahl method. His results wed a value of 12 mmole/l. (M/84.5) of cephalin.

The carrier transport with cephalin is blocked at high and low values of *p*H. en lecithin was used as the carrier, there is no transport between NaCl and l in acid *p*Hs, but at high *p*Hs Na and K exchange rapidly from KOH and OH solutions (*p*H 11.9) despite some hydrolysis of the phospholipid. The erent behavior of lecithin to cephalin at different *p*H's can be explained by internal salt linkage between the positively charged substituted nitrogen up and the negatively charged phosphate group. This blocks the addition NaCl or KCl to the phospholipid in acid *p*Hs, but in alkali solution the link- is weakened. Therefore K^+ and Na^+ ions in the respective salt solutions build salt linkages with the lecithin molecule, and K^+ and Na^+ ions can hange at high *p*Hs.

The addition of alkyl phosphates or amines (dioctyl acid pyrophosphate, noisoamyl orthophosphate and stearyl amine) to 1-pentanol either sepa- ely or in equimolecular combination produced no exchange of K^+ and Na^+ ler the same conditions as with cephalin and lecithin. Similar negative ilts were obtained with dodecyl β-alanin in the 1-pentanol. These results phasize that a dual salt linkage is essential with the amphoteric compound before an ionic exchange can take place. The ionic charges must be separat as the amino acid confirms the experiments with ethanolamine and ser cephalins, which are independent of the presence of the carboxyl group.

The effect of temperature on the carrier transport of NaCl against K through a M/100 cephalin 1-pentanol membrane was also studied. Betwe 24° and 42° C. the ionic exchange is practically unchanged. This indica

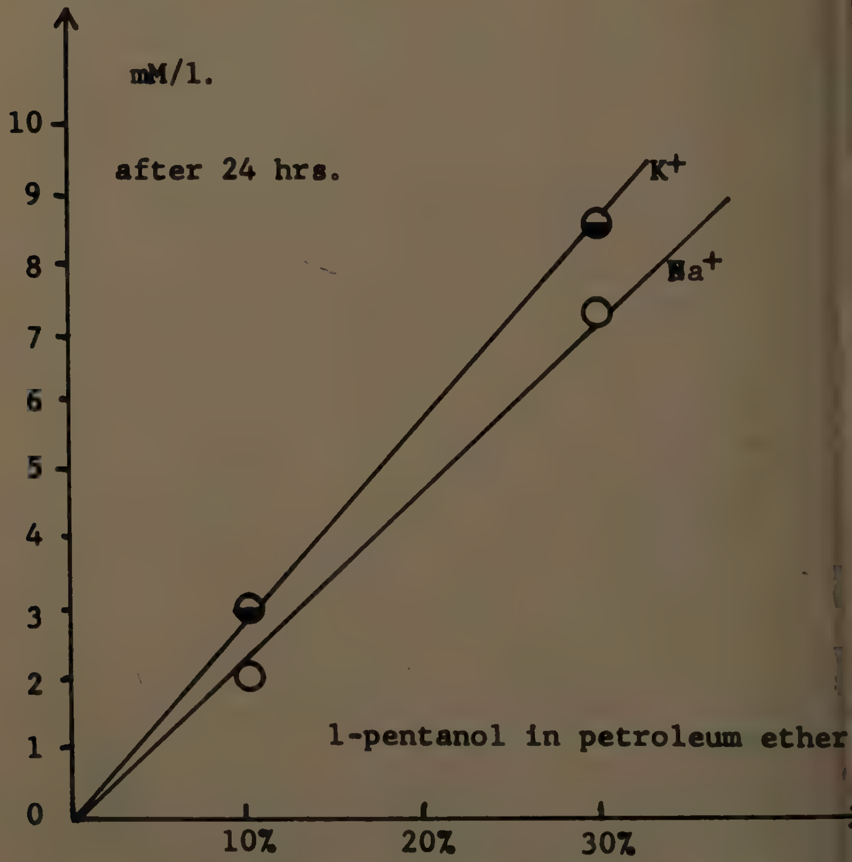


FIGURE 5. Na^+ and K^+ transport versus percent of 1-pentanol in petroleum ether.

that no diffusion occurs with the carrier transport mechanism and that number of cephalin molecules at the oil/water interface remains constant o this temperature range, or that these two processes change complementar (FIGURE 7).

The ionic exchange between Na^+ and K^+ ions of 2 equimolecular NaCl KCl solutions through a M/100 cephalin 1-pentanol membrane for increas salt concentrations was determined (FIGURE 8). The amount of sodium i exchanged against potassium ions increased rapidly up to 50 mmole/l. then slightly decreased. This shows that this salt concentration produces

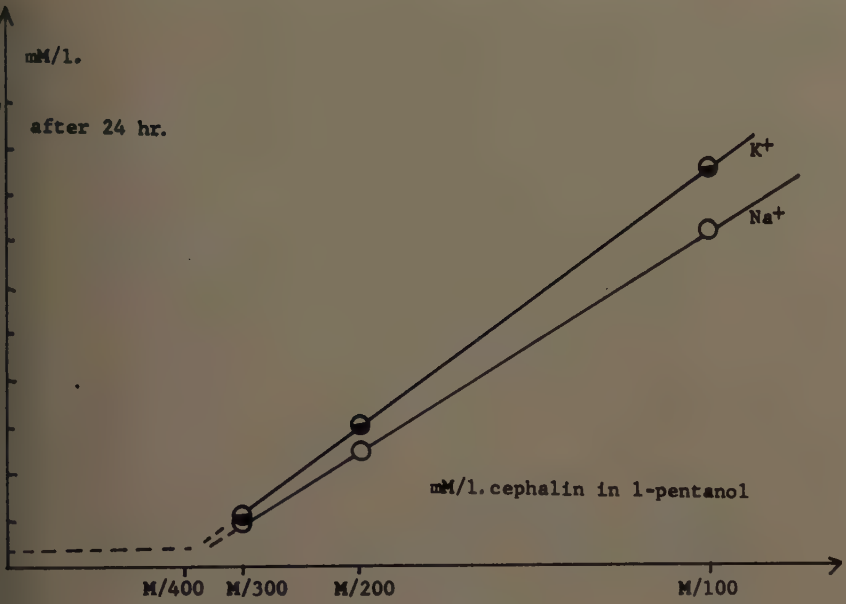


FIGURE 6. Influence of the cephalin concentration in 1-pentanol on the Na^+ and K^+ rier transport.

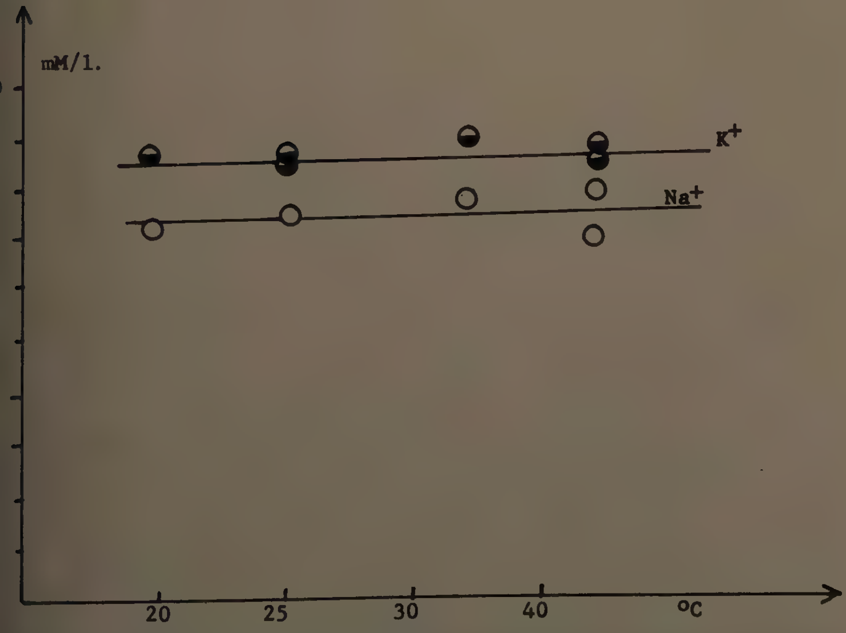


FIGURE 7. Effect of temperature on the carrier transport of 50 mM/l. NaCl opposed to mM/l. KCl through M/100 cephalin 1-pentanol.

maximum ionic surface concentration for the attachment to the availab
cephalin molecules at the oil/water interface. The slight decrease may be d
to discharge of the ionic groups in the cephalin molecule.

An ionic pump model against concentration gradient. It was found possib
to realize an ionic pump model by opposing 50 mmole/l. of NaCl against 1
mmole/l. of a NaCl-KCl mixture across a M/100 cephalin 1-pentanol solutio
In this set of experiments, the ratio of NaCl to KCl varied from 0 mmole
KCl—100 mmole/l. NaCl to 100 mmole/l. KCl—0 mmole/l. NaCl. For ea
of these NaCl-KCl mixtures the changes of concentration were measured f

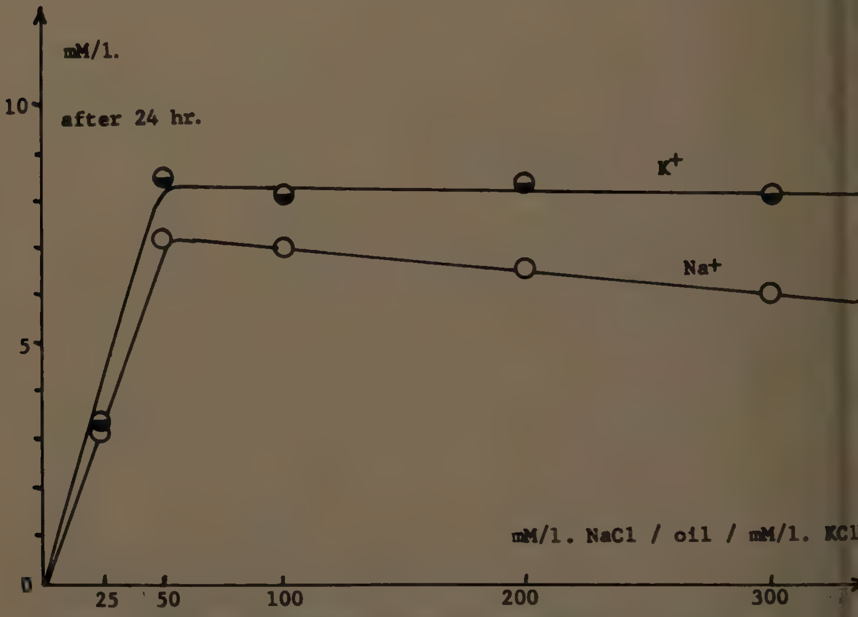


FIGURE 8. Influence of increasing identical concentrations of NaCl opposed to K
through M/100 cephalin 1-pentanol (at 30° C) on the carrier transport.

Na^+ and K^+ ions in the 2 aqueous compartments over a period of approximate
30 hours.

In FIGURE 9 the concentration, after 24 hours, of K^+ ions in the NaCl co
partment and the increase over the initial NaCl concentration in the NaCl-K
compartment have been plotted for several NaCl-KCl ratios. It may be se
that the increase of Na^+ concentration in the NaCl-KCl side is compensat
by approximately the same amount of K^+ ions exchanged in the NaCl compa
ment. It has been found that Na^+ ions can move against the concentrati
gradient of Na^+ by adding in one of the two NaCl compartments, other sa
with exchangeable ions. It was concluded that an ionic pump model agai
concentration gradient can be realized by adding an exchangeable ion into o
of the two compartments containing an identical salt. The shape of the cur
of FIGURE 9 suggests that the exchangeable and the nonexchangeable i

npete for the carrier molecules at the oil/water interface. The effect of hangeable surface active ions will be investigated in a subsequent publica- n.

The influence of anions upon the Na^+ and K^+ transport has been investigated

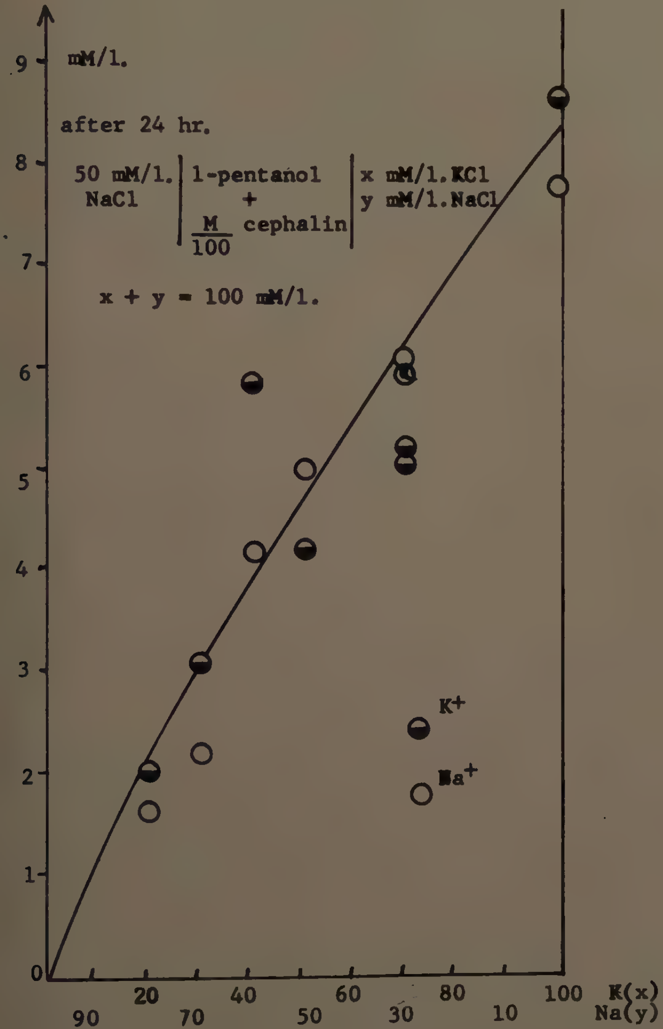


FIGURE 9. Sodium migrating against its concentration gradient on exchange against assium.

opposing 50 mmole/l. sodium concentration to 50 mmole/l. of K^+ ions ncentration. It has been shown in a previous publication[1] that carbonates d sulfates are poorly diffusible compared to chlorides and that, furthermore, y do not undergo ionic exchange with cephalin or lecithin through alcohols.

It is shown further that Na^+ and K^+ can exchange independently of the n exchanging anions. It can be seen in TABLE 3 that the presence of sulfat carbonate greatly enhances the Na^+ and K^+ ionic exchange as compared to chlorides. This is even more dramatic in the case of the divalent anions si they do not, on their own, diffuse or exchange. The reason for this phen enon becomes apparent when one considers the case of the ionic excha through a petroleum ether $-M/100$ cephalin membrane being marke enhanced by the addition of 1-pentanol. As previously explained,[1] the pentanol molecule is holding the cephalin at the oil/water interface by comp formation. In the case of the carbonates and sulfates, two cephalin molec are anchored at the interface as compared to one with the chloride, thus abling a much more rapid ionic exchange to take place between the Na^+ K^+ ions. The enhancement shown by NaCl-KCl in 1-BuOH + cephalin compared to 1-pentanol cephalin can be readily explained by the diffusio the chlorides through 1-BuOH but not through 1-pentanol.

TABLE 3
INFLUENCE OF THE ANIONS UPON NA^+ AND K^+ TRANSPORT THROUGH 1-BUTANOL AND 1-PENTANOL AFTER 24 HOURS (30° C.)

Systems	1-BuOH + M/100 cephalin		1-BuOH		1-Pentanol + M/10 cephalin	
50 mM/l. as Na^+ or K^+ in each opposing compartment	K^+ in mM/l.	Na^+ in mM/l.	K^+ in mM/l.	Na^+ in mM/l.	K^+ in mM/l.	Na^+ in mM
NaCl-KCl	15.6	13.4	8.0	6.6	8.7	7.6
NaCl-K_2CO_3	17.8	16.0				
Na_2CO_3-K_2CO_3	19.3	18.6	1.8	1.6		
NaCl-K_2SO_4					9.0	10.1
Na_2SO_4-K_2SO_4	18.7	12.3				

Inhibitors. The carrier mechanism with cephalin is completely inhibited a small concentration of uranyl nitrate. A reduction in carrier transport also be brought about by addition of M/3000 curare to the salt solutions. transport is diminished by half. These compounds are well known biolog inhibitors and it is hoped to work out biological analogies with these syste

Acknowledgments

We thank P. Duby for experimental help and discussions.

Summary

A study has been made (1) on the influence of the chemistry of the n aqueous liquid membranes on the migration of salts, ions, and the water tra port through these membranes; (2) on the carrier mechanism of the migrat of anions and cations through diffusion blocked nonaqueous liquid membra in the presence of amphoteric phospholipid carriers; and (3) on the migrat of ions against their concentration gradients by an ionic exchange mechan taking place at the oil/water interfaces.

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AMINE BUFFERS AND YEAST*

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In walled cells, such as those of yeast, no electrolytes are required in t medium to balance the cellular osmotic pressure. The cell wall is able to re swelling even when the cells are suspended in distilled water. The ionic co position of the medium is important, however, because it is a major factor determining the electrolyte composition of the cell, and because it influen the properties of the cell membrane and of metabolic functions associated w the periphery of the cell.[1] The ionic composition is a major consideration selecting buffers for different types of experiments. The problem is part ularly difficult in the case of yeast cells for two reasons: (1) they can tolera a wide range of *p*H values in the extracellular medium, from below *p*H 2.0 over *p*H 9.0; (2) they normally secrete large quantities of acid, during acti metabolism and, in some circumstances, can absorb acid.

The usefulness of a buffer depends not only on its *p*K, but also on wheth or not it is "inert" with respect to the biological system under study. particular, in yeast, the cations must serve as inactive substitutes for ions su as K^+ or Na^+, which play an important physiological role. Amine buffe triethylamine (TEA) in combination with organic acids, and trisaminoh droxymethane (Tris) have been especially useful. Before examining the effe of TEA and of Tris in yeast systems, however, a brief discussion of the probl of the buffer anions is necessary.

The Anions

The yeast cell is relatively impermeable to anions but permeable to undis ciated acids. For this reason, monocarboxylic acids are of little use as buf anions in the acid *p*H range. For example, pyruvate is not respired at neut *p*H values, but as the *p*H is reduced to levels at which undissociated acid formed, the acid penetrates and is respired at increasingly high rates.[2] T same thing is true of acetate and lactate. For these reasons, dicarboxy acids such as tartrate and succinate were chosen as buffers. They buffer ove wider range (2.0 to 6.0) and do not penetrate the cell unless the *p*H is ve low.[3]

In the neutral range, bicarbonate has been used, but it is inconvenient many types of study. Phosphate is of little use as a buffer because it pla such a central physiological role. Given sugar, the cell absorbs phosphate the form $H_2PO_4^-$. As a result, the medium becomes more alkaline.[4] T phosphate absorbed may, in turn, influence other cellular functions. In so experiments at neutral *p*H the problem of buffers was avoided by use of a *p* stat† that maintains constant *p*H by injection of triethylamine chloride in the yeast suspension.

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* This study is based on work performed under contract with the United States Ato Energy Commission at the University of Rochester Atomic Energy Project, Rochester, N.

† International Instrument Corporation, Canyon, Calif.

Cation Binding to the Cell Surface

The outer surface of the yeast cell contains anionic groups that can reversibly d cations of the medium.[5] In FIGURE 1, the binding curve for Mn^{++} is ressed in terms of a derivation of the mass law,

$$\frac{(MY)}{(M)} = \frac{fmYt}{K_1} - \frac{fm(MY)}{K_1}$$

ere MY is bound Mn^{++}, M is free Mn^{++}, Yt is the total number of binding s, K_1 is the affinity constant, and fm is the activity coefficient of Mn^{++}. o binding groups are represented by the two slopes. The steep slope has n identified with binding by phosphoryl groups and the shallow slope with boxyl groups.[6]

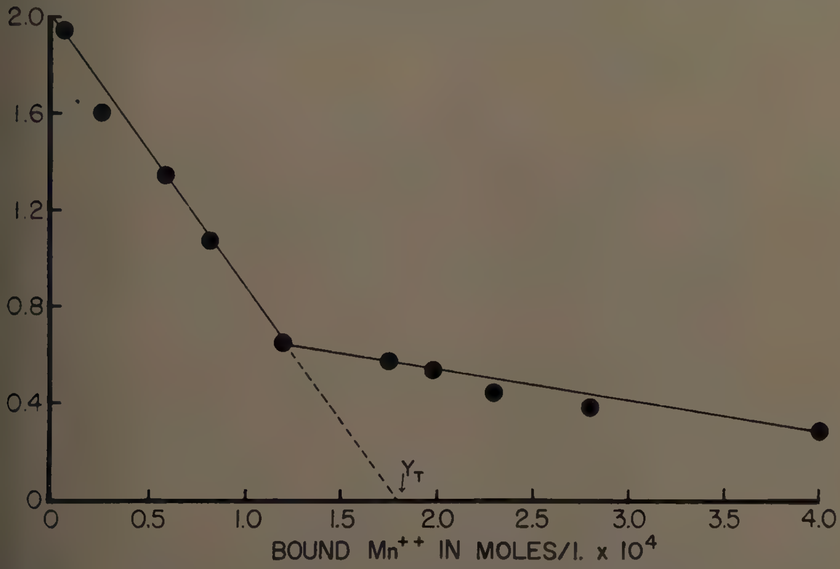


FIGURE 1. A mass law plot of the Mn^{++} uptake by yeast cells. Reproduced by permission Academic Press, Inc.[5]

TABLE 1

RELATIVE BINDING AFFINITIES OF CATIONS FOR GROUPS ON THE CELL SURFACE

UO_2^{++}	330	Cu^{++}, Hg^{++}	1.0
Ba^{++}	10	Li^+, Na^+ K^+, Rb^+, Cs^+	0.003 to 0.005
Zn^{++}	3		
Co^{++}, Mg^{++} Ca^{++}, Sr^{++}	1.5	TEA$^+$	
Mn^{++}	1.0	Tris$^+$	0.001

A series of competition experiments with Mn^{++} has allowed estimates of the tive affinities of the phosphoryl groups for different cations (TABLE 1). $_2^{++}$ has an especially high affinity compared with other bivalent cations.

Monovalent cations such as Na^+ and K^+ are definitely bound, but the affini is relatively low. Within the limits of error of the procedure, TEA^+ and Tri are not bound, although in TABLE 1 they are reported with relative affinities less than 0.001. They can therefore be considered as "inert cations" wi respect to the binding sites.

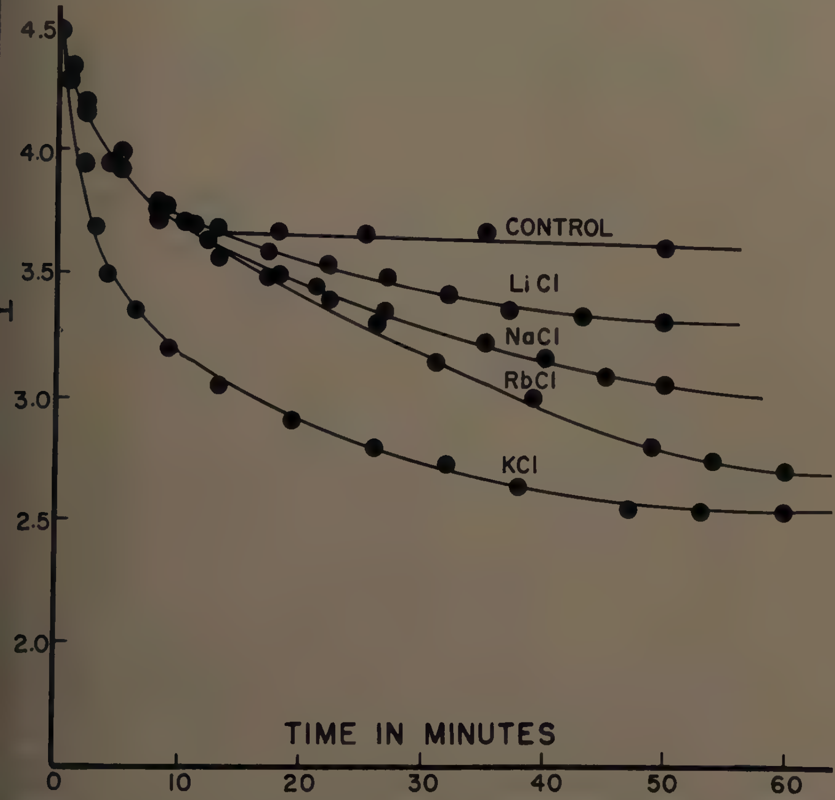
The physiological role of the binding sites is not clear. For example, th are not involved as a first step in the entry of K^+ into the cell,[7] nor is a part ular cation-binding required for metabolism, the rate of fermentation being t same, if Na^+, Ca^{++}, or Mg^{++} is bound. If, however, the sites are filled wi heavy metal cations such as UO_2^{++}, the cells can no longer take up suga perhaps because access of the sugar to the site of its transport across the c membrane is blocked.[8]

Ion Exchanges

Many experiments with yeast have been done in unbuffered media, the being simply allowed to seek its own level. For example, if well-starved ce are suspended in distilled water, the *p*H of the medium will slowly become mo alkaline, due to an outward leakage of K^+ in exchange for H^+.[9] If glucose added, the *p*H will drop to about 3.5 (FIGURE 2) due to secretion of succin acid. If K^+ is added as well (in the form of KCl) the *p*H drops much mo rapidly and to much lower levels (2.6 in this case).[3] The final value for t *p*H is dependent on the K^+ concentration and on the yeast concentration Values as low as 1.7 have been observed.[10] The shifts in *p*H with K^+ a glucose are not due in this situation to succinic acid secretion, but results fro a stoichiometric exchange of K^+ and H^+. The cell specifically transports from outside to inside. Because the membrane is relatively impermeable anions, the inward movement of K^+ is balanced by an outward movement H^+ produced by metabolic reactions. Other cations can substitute for K^+ a degree; Rb^+ is best, whereas Na^+, Li^+, and Cs^+ are relatively ineffecti Conway has quantitatively determined the specificity of the K^+-transporti systems and reports the ratios 100:42:7:4:0.5:0:5 for K^+, Rb^+, Cs^+, Na Li^+, and Mg^{++}.[11]

In the experiment of FIGURE 2, TEA^+ or $Tris^+$ did not induce any acid pr duction beyond the control. On this basis, neither can participate to an a preciable extent in the exchange reaction for cellular H^+. In another stud however, some exchanges were detected, but only if the extracellular *p*H w above 4.5. A K^+-free solution was passed through a column of yeast cells, a the eluates were collected with a fraction collector.[12] Under such circu stances, a small efflux of K^+ is observed that is balanced by the entrance in the cell of an equivalent amount of H^+. The cation exchange proceeds fast at lower *p*H because of the larger gradient of H^+ into the cell (FIGURE 3). addition, the K^+ efflux is much increased by the presence of glucose. Oth cations added to the medium that can enter the cell would be expected to i crease the outflow of K^+ by exchange. At *p*H values below 4.5, the presen of TEA^+ at concentrations of 0.02 *M* have no effect on the outflow of K^+. *p*H values above 4.5, however, the outflow of K^+ is markedly increased (cur marked "glucose and buffer"). A generalized change in the selectivity of t mbrane seems to occur at pH 4.5, for cations other than TEA^+ (Na^+ and H_4^+) have a similar effect in increasing the outflow of K^+. That the counter-s actually enter the cell in exchange for K^+ is demonstrated by direct analysis the cells.

TEA$^+$ does not participate in, nor influence other ion transporting systems yeast. Those studied include the phosphate-transporting system[4] and the g^{++}-transporting system.[13]



IGURE 2. Changes in the pH of the medium during fermentation of glucose, as influenced cations. Reproduced by permission of Academic Press, Inc.[3]

Metabolism

he metabolism of yeast cells is especially sensitive to the cationic environ-nt, especially to H^+ and to K^+. The use of physiologically inert buffers is refore of primary importance. Investigations have covered a wide range H, from 2.0 to 9.0, too wide for any one buffer system. As in the experi-nts on ion exchanges, a combination of TEA^+ succinate and tartrate was d in the range 2.0 to 6.0, TEA^+ with bicarbonate or phosphate in the neutral ge, and Tris or TEA^+ barbital in the range 8.0 to 9.0.

he pH activity curve for fermentation in the presence of TEA^+ or Tris$^+$

follows a biphasic curve with peaks at *p*H 4.5 and 8.0 and a valley at *p*H 7.7
If K$^+$ is added to the system, the peaks and valleys are obliterated and th
metabolism follows a broad plateau over the range *p*H 3.0 to 8.0 (FIGURE 4
In the acid range (*p*H 2.5 to 3.5), the yeast is self-buffering, and a varie
of cations could be tested against a cation-free (except for H$^+$) baseline. E
cept for K$^+$, none of the cations, including TEA$^+$ or Tris$^+$ had any effect on th

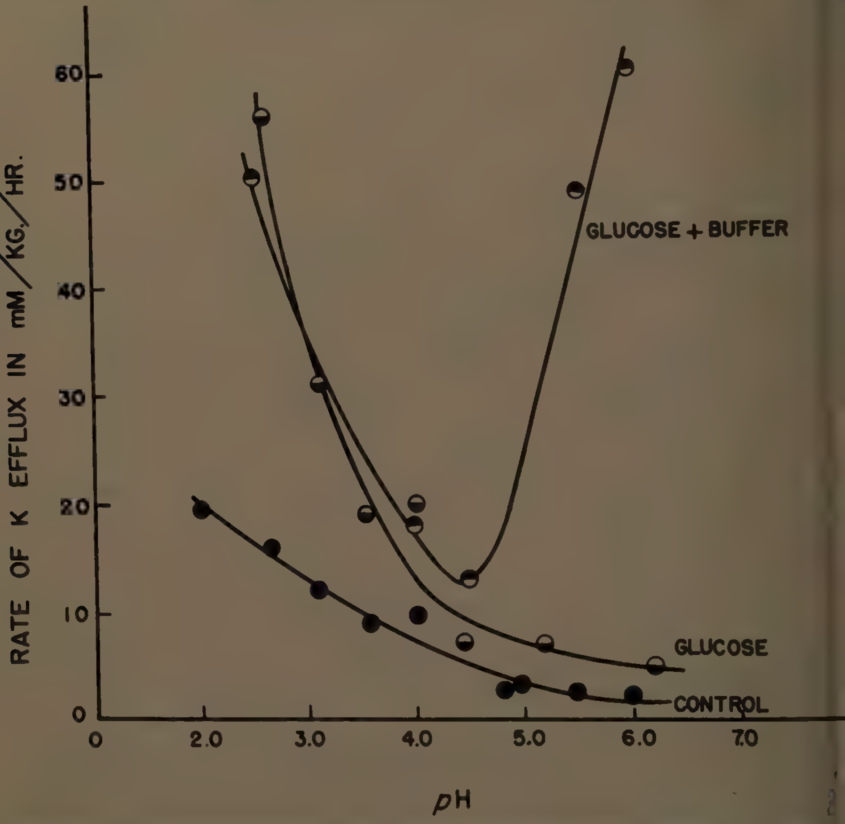


FIGURE 3. The effect of *p*H on the K$^+$ efflux in the presence and absence of glucose a
buffer (triethylamine with succinate and tartrate). Reproduced by permission of the *Jour*
of Cellular and Comparative Physiology.[12]

rate of metabolism. At higher *p*H values a stable *p*H could be maintain
only by the use of buffers, so comparisons with cation-free media were difficu
At *p*H 6.0 (with succinate, tartrate buffer), TEA$^+$ gave a very low rate
metabolism. With either K$^+$ or NH_4^+ the metabolism was stimulated to
maximal level (about 50 µl./mg./hour), as shown in TABLE 2. With Na$^+$ t
rate was intermediate. The low value with TEA$^+$ might result from an
hibitory action of this ion. On the other hand, TEA$^+$ may have no effe
but a high rate of metabolism can proceed only if K$^+$ or NH_4^+ are prese
The latter alternative seems more acceptable. First, at *p*H 3.5, TEA$^+$ had
effect. Second, without buffer and with dilute yeast suspensions, the

ds at about *p*H 6.0 for sufficient time to obtain a few readings. The rates metabolism are about the same as those with TEA present.

n the alkaline range (*p*H 8.0 to 9.0) no baseline (absence of cations) could obtained, but some comparisons of buffers were made (TABLE 3). Na-barbi-

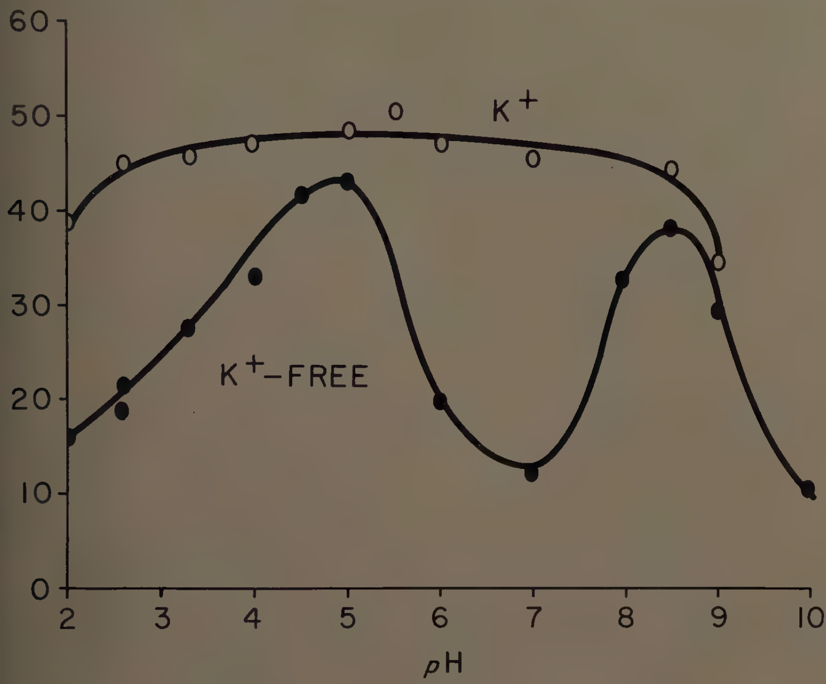


FIGURE 4. The effect of *p*H and of K+ on the rate of fermentation.

TABLE 2
EFFECTS OF CATIONS ON THE RATE OF FERMENTATION AT DIFFERENT VALUES OF EXTRACELLULAR *p*H

Ions	*p*H		
	2.5	3.5	6.0
None	14.3	31.0	—
TEA+	14.5	30.5	14.4
Tris+	14.0	31.0	—
K+	54.1	53.0	47.6
NH_4^+	15.3	32.2	50.2
Na+	15.5	31.3	32.6
Mg++	—	29.8	27.0

ates of fermentation are in microliters of CO_2/mg. wet wt./hour.

gave somewhat higher rates than Tris and than TEA barbital, perhaps due he stimulating effects of Na+. However, the buffers themselves are not pletely "inert" because with increasing concentrations, the rate of respira- was diminished.

TEA^+ Resins

From the data of FIGURE 4 and of TABLE 2, it is obvious that the rate metabolism of yeast is especially influenced by extracellular K^+. Under or nary circumstances, the full extent of the K^+-dependence cannot be determi because some K^+ leaks into the medium from the cells (in which the concent tion is as high as 0.2 *M*). In fact, such leakage can result, in some circu stances, in a build-up of K^+ to the extent of 2 to 3 $\times$ 10^{-3} *M*, sufficient to ha an appreciable effect on metabolism. K^+-free medium can be maintained a continuous flow of medium through a column of cells, as described previou in connection with the data of FIGURE 3. Another method involves the of a cation exchange resin that has been saturated with TEA. Such a re shaken with the yeast cells will continuously "soak up" K^+ released by cells, releasing TEA^+ in exchange.

TABLE 3

EFFECTS OF BUFFERS ON THE RATE OF RESPIRATION OF YEAST AT *p*H 8.5

Buffer concentration	Na^- barbital	Tris	TEA barbital
(*M/l.*)			
0.02	20.5	17.4	—
0.07	18.9	16.8	15.2
0.15	16.0	11.7	—

Rates of respiration are in microliters of O_2/mg. wet wt./hour.

TABLE 4

EFFECT OF TEA-RESIN TREATMENT ON THE RATE OF FERMENTATION OF YEAST

	*p*H 3.5	*p*H 5.5
Control	30.5	18.5
Control + K^+	53.0	49.5
Resin-treated	28.5	6.0
Resin-treated + K^+	45.0	45.5

All values are in CO_2 production in µl./mg./hour. Yeast was shaken with TEA-resin 2½ hours; K^+ concentration, 0.02 *M*.

TABLE 5

ACTION OF BUFFERS AND EXCHANGE RESINS ON THE RATE OF FERMENTATION OF IRRADIATED YEAST CELLS

	Control	Irradiated*	Change (%)
K^+-buffer	56	63	+12
TEA buffer	53	46	−13
TEA resin and TEA buffer	28	16	−43

* Irradiation was 90,000 of 250 kv. X ray, in oxygenated water. The buffers and re treatment refer to the postirradiation period, during which the metabolism was measure

Some of the effects of TEA-resin treatment are demonstrated in TABLE

h the rate of metabolism measured at pH 3.5, a small decrement in the
of fermentation was observed, but at pH 5.5 the rate was reduced to one
d of normal. The reduction in rate is attributable to a loss of K^+, because
ition of this cation almost completely abolishes the effect. The TEA resin
used in these experiments to establish a lower baseline level for metabolism
he absence of K^+, and to unmask thereby the role of the cation. Some
caution is necessary, however, because prolonged treatment (5 to 6 hours)
lts in such a great loss of cellular K^+ that the metabolism is decreased to
levels (regardless of pH), and it cannot be restored by K^+.

striking use of the TEA resin to unmask the effects of K^+ is given in TABLE
om the data of Bair and Stannard.[14] Cells treated with X ray and then
ed in K^+ buffer showed little effect. Tested with TEA buffer, about 13
cent inhibition was measured. With TEA resin present, the inhibition
much larger. The irradiation does not damage the metabolic system,
it does injure the cell membrane so that K^+ cannot be retained. Under
ditions such that the K^+ is continuously removed from the medium, its
is reflected in a diminished metabolism.

Summary

EA$^+$ is a relatively inert cation with respect to the yeast cell. Its use in
fers has allowed investigation of the effects of extracellular pH on functions
h as metabolism and ion transport. Furthermore, it has provided a base-
for studying the role of extracellular cations, especially K^+. In a contin-
s flow system (cell column), TEA buffers allow the maintenance of K^+-free
tions despite leakage from the cells. The same situation can be obtained
ell suspensions by the use of a TEA$^+$-cation exchange resin. Tris has also
n useful in studies in the alkaline pH range, but this substance may not be
rely inert with respect to cellular function.

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PERMEABILITY OF CELL MEMBRANES TO AMINE BUFFER AND THEIR EFFECTS ON ELECTROLYTE TRANSPORT[*]

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Nahas and his associates[1] have reported that the survival of dogs in exp
mental respiratory acidosis is prolonged by the administration of an orga
amine, tris(hydroxymethyl)aminomethane (THAM). These authors h
proposed that THAM may be particularly effective because the organic
may enter cells and act as an intracellular buffer. This view is supported
its apparent volume of distribution in the whole animal.[2] The present rep
is concerned with evidence obtained from simpler *in vitro* systems that indic
that organic amines can penetrate cells and alter rates of cellular proces
Volume changes of human erythyrocytes in suspensions containing organic am
buffers have been investigated, as well as the influence of these substance
the short circuit current in frog skin. In so far as we are aware, the perm
bility of erythrocytes to aliphatic amines has not been reported, altho
pyridine has been found to penetrate red cells.[3]

Methods

Fresh or 1-day-old defibrinated human blood was centrifuged, and red c
were washed twice with isotonic NaCl. Approximately 2 ml. of washed c
were suspended with 98 ml. of saline. Two ml. of this suspension were pla
in 22 × 175 mm. Pyrex test tubes containing 20 ml. of a test solution. Opt
density (O.D.) was measured in a Lumetron colorimeter at a wave length
650 mμ. The suspensions were stirred during the measurement by means
Teflon-coated bar activated by a magnetic stirring bar positioned directly un
the test tube. The essential procedure followed is a classic one used for ma
years.[4,5]

Standard curves were obtained by measuring optical densities of red
suspensions in 0.001 M phosphate-buffered (pH 7.4) NaCl solutions hav
different tonicities. A linear relationship was obtained when the reciproca
optical density, 1/O.D., was plotted against the reciprocal of osmotic conc
tration, $1/c$ (FIGURE 1). This relationship has been observed before,[6] and si
red cell volume, V, and osmotic pressure have been shown to be reciproca
related,[7] it appears that the empirical relationship $1/\text{O.D.} = K1/c + \beta$, bea
direct relationship to osmotic law, $V = K1/c + b$. K is a proportionality
stant and b is the so-called osmotic dead space. These considerations h
been discussed thoroughly in papers on erythrocyte[6] and mitochondrial[8]
ume. The basis for the relationship between light transmission and cell
ume is believed to be due to the difference between refractive indices of
pended cells and of the medium.[6,8,9]

In frog skin experiments, skin from the thigh region of *Rana pipiens*
placed between Lucite blocks containing chambers that permitted both si

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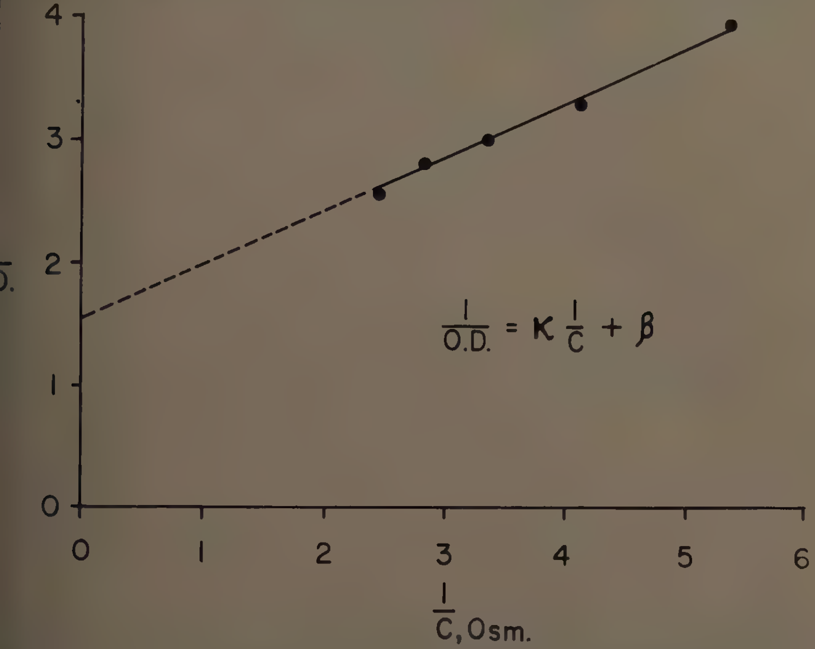
[*] The investigation reported in this paper was supported in part by Grants H-4155
H-4740 from the National Heart Institute, Public Health Service, Bethesda, Md.

segment of skin to be bathed. The sulfate Ringer's used as the fluid me-
contained 100 mM Na, 5 mM K, 52.5 mM SO_4, 22.5 mM THAM or
zole, and 20 mM mannitol per liter of solution. Sulfate Ringer's buffered
phosphate contained the same concentrations of Na and K, 29.5 mM SO_4,
M HPO_4:H_2PO_4, and 40 mM mannitol per liter of solution. Although
otal concentration as prepared was 200 mM, the mOsm. concentration by
reezing point method was 165, 167, and 162 mOsm. for THAM-, imidaz-
and phosphate-buffered media. The discrepancy appears to be due chiefly
e low activities of Na_2SO_4 and Na_2HPO_4.[10] Measurements of skin po-



URE 1. The linear relationship between the reciprocals of optical density and of os-
concentration. The y-intercept β may be regarded as some function of osmotic dead

al and short circuit current were carried out essentially by the procedure
sing and Zerahn.[11]

several erythrocyte experiments, solutions were prepared that were
ly more concentrated than those desired, osmolalities were determined by
ng point in a Fiske osmometer, and appropriate dilutions were made to
the desired concentrations.

utions of 0.15 M tris(hydroxymethyl)aminomethane (Sigma), 0.10 M
zole (Eastman), and 0.15 M 2,4,6-trimethylpyridine (Eastman) were
to have pK values of 8.1, 6.9, and 7.2 respectively.

Penetration of Organic Amines into Human Erythrocytes

lassic procedure in demonstrating solute penetration into cells is the meas-
ent of increases in cell volume as solute particles enter followed by water.

Using the photometric method, cell volume increases were observed in a our erythrocyte experiments with THAM (FIGURES 2 to 5) so that this org base does appear to penetrate the cell membrane of human red cells.

As is generally the case with weak acids and bases, THAM molecules present in both un-ionized and ionized forms in solutions with a pH near pK of the organic base. A general finding has been that the undissoci form of a weak acid or base is the principal penetrant.[12] The same conclu may be made with regard to THAM since the rate of entry appears to be gre at higher pH, where the undissociated form is present in higher concentra (FIGURE 2). This result may be contrasted with volume effects of pH wh

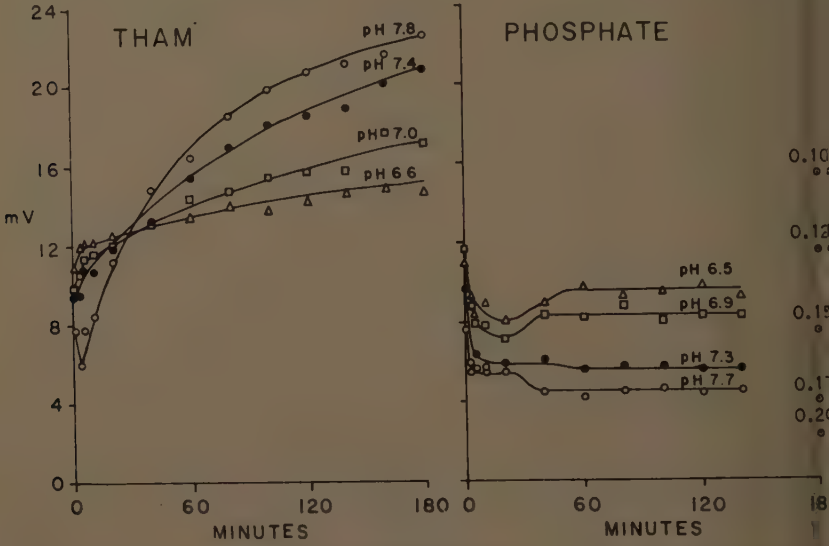
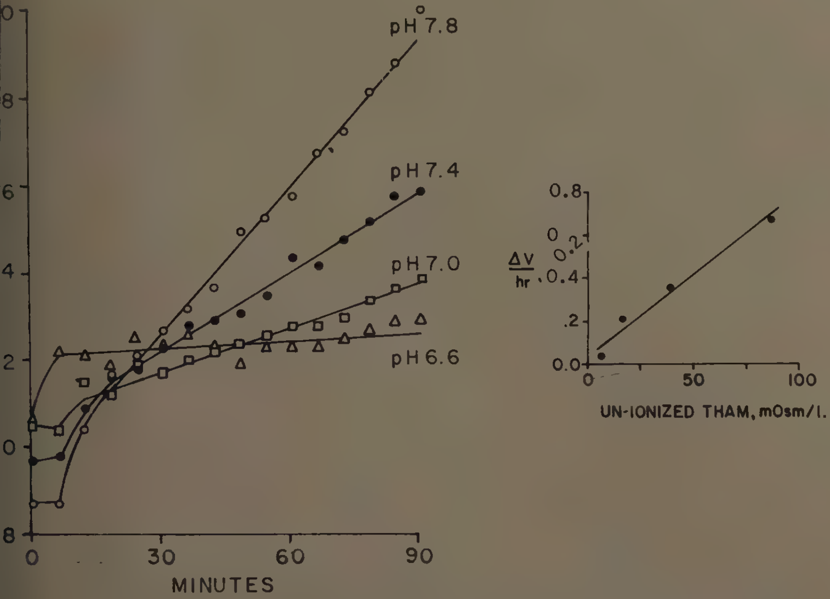


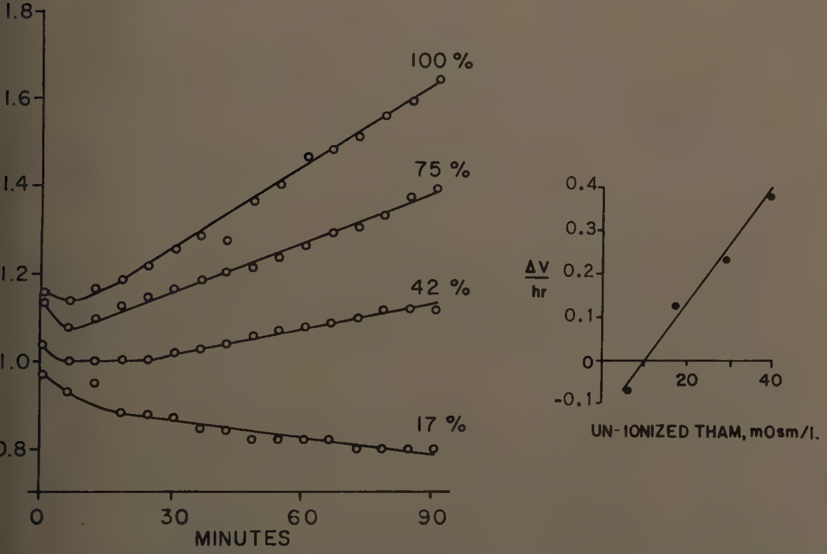
FIGURE 2. Light transmission of red cell suspensions in THAM and phosphate buffe functions of time and of pH. Isotonic (300 mOsm.) solutions were calculated by taking account the proportion of ionized and un-ionized species at a particular pH. In this ex ment only, readings were taken from a millivoltmeter. The values at the extreme righ of NaCl solutions at different concentrations buffered with phosphate at pH 7.4.

nonpenetrating buffer substance is employed. When phosphate is the so (FIGURE 2), cell size is smaller at higher pH since the number of colliga particles within the cell decreases as the number of multivalent hemogl anions increases.[13] It may be noted that the initial cell volumes in the TH series follow this relationship but as THAM penetrates, the curves cross due to differences in rate of volume change at different pH.

In subsequent studies with THAM, the external concentration was raise 413 mOsm. so that a greater number of points could be derived within linear range of the standard curve and thereby allow the results to be expre in terms of volume change. The ratio $(V - b):(V_0 - b)$ is the ratio of os ically active volumes at time t and at time 0 and may be obtained by equa it to the ratio $\frac{(1/\text{O.D.})_t - \beta}{(1/\text{O.D.})_0 - \beta}$, where β is a function of osmotic dead space



GURE 3. Volume change of red cells suspended in THAM as a function of *p*H, expressed e ratio of osmotically active volumes at time *t* and at time 0. Solutions were all 413 n. At the right, the rate of change of volume (following the initial transient effects) of curve is plotted against the concentration of un-ionized THAM.



GURE 4. Volume change of red cells suspended in THAM as a function of THAM ntration. Different proportions of 413 mOsm. THAM and 413 mOsm. NaCl were l; for example a 75 per cent THAM solution was a mixture of 3 parts THAM and 1 NaCl. At the right, the rate of volume change (following the initial transient effects) h curve is plotted against the concentration of un-ionized THAM.

may be obtained from the *y* intercept in the standard curve (FIGURE 1). . FIGURE 3 also, it is apparent that volume increases are greater at higher When the rate of change in volume is plotted against un-ionized THAM centration, a linear relationship is seen (FIGURE 3). These results indi

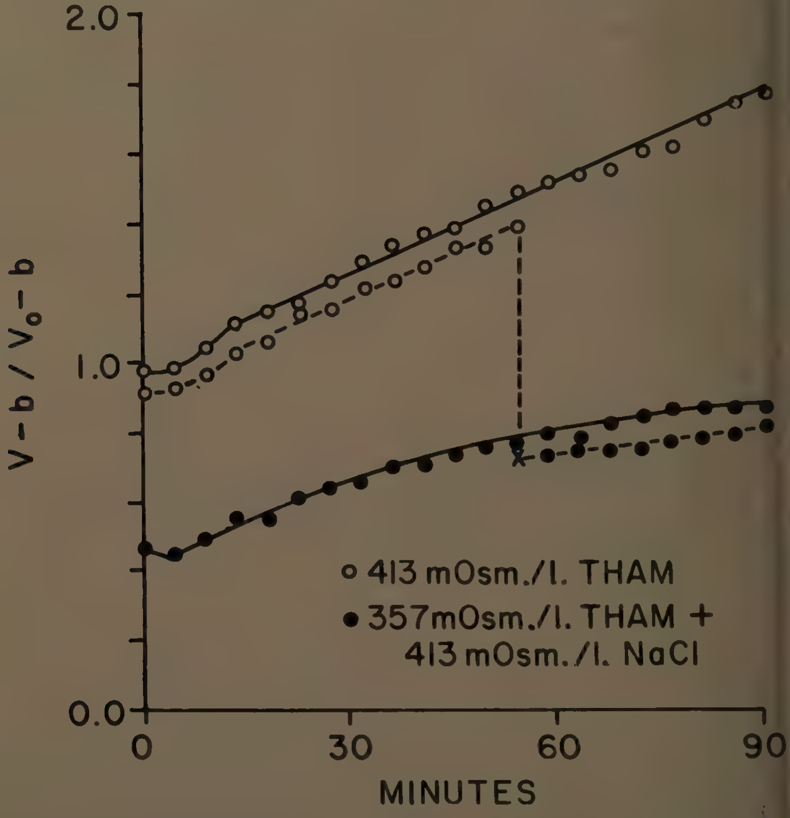


FIGURE 5. Osmotic reversal. To 1 of 2 sets of tubes containing 413 mOsm. TH concentrated NaCl was added to make the final concentration 413 mOsm. NaCl + mOsm. THAM. The anticipated volume following reversal is marked by an *x*. The curve is from a third set of tubes containing 413 mOsm. NaCl + 357 mOsm. THA time 0. Volume calculations of cell suspensions with the added volume of NaCl were from a standard curve obtained from cell suspensions at different tonicities made up t same total volume.

only roughly the true penetration rate since internal ionization and concomi effects on cell volume cannot be readily estimated.

Solutions with different THAM concentrations at a single *p*H (7.4) prepared by mixing different proportions of 413 mOsm. THAM and of mOsm. NaCl. As shown in FIGURE 4, greater change in volume occurre higher THAM concentrations and the rate of change in volume appears to a direct relationship to un-ionized THAM concentration. Similar results obtained at 300 mOsm. concentrations. Thus, in addition to showing tha me increases are related to undissociated THAM concentration, these re- also demonstrate that the *p*H findings (FIGURES 2 and 3) can be explained rms other than a *p*H effect on the permeability of the membrane to THAM. curve obtained with 17 per cent THAM was identical to that obtained with phate-buffered 413 mOsm. NaCl so that an associated event with hyper- solutions appears to be leakage of cell solute.

n experiment that serves to distinguish between penetration and a possible kening of the cell membrane, such as is caused by lysins, is the osmotic rsal experiment.[8] The classic experiments in this regard are those in which swollen by hypotonicity can be reversed to normal size by hypertonic NaCl tions whereas cells swollen in the presence of saponin fail to reverse since membranes are no longer semipermeable.[14] In FIGURE 5 the effect of add- concentrated NaCl during the course of cell volume increase is shown. If cell membranes were unaffected by THAM, it may be anticipated that the volume should be reduced to about one half when the medium is made roximately two times as concentrated. It may be noted that the curve drop to a point near the theoretical, indicating that the integrity of the membrane was not particularly affected by the presence of THAM. It found experimentally that the time allowed for reversal was sufficient for otic equilibrium to have taken place. In addition, the curve for a solution was initially 413 mOsm. NaCl and 357 mOsm. THAM may be seen to roach the point of equilibrium reversal, 413 mOsm. NaCl.

wo other organic amines were studied in 300 mOsm. solutions, and evidence enetration was obtained, the principal variation from the THAM results g one of kinetics. With 2,4,6-trimethylpyridine, an organic base having same molecular weight as THAM, an aromatic nucleus, and a *p*K of 7.2, rate of volume change was so rapid that the results are expressed in terms emolysis times. Hemolysis was observed in roughly 23 sec. with isotonic ions of this organic amine buffer at *p*H 7.4. With imidazole, hemolysis s of 35, 47, 104, and <600 sec. were obtained at *p*H 7.8, 7.4, 7.0, and 6.6, ectively. No hemolysis was observed over a period of 90 min. when cells suspended in solutions that were isotonic with respect to both imidazole NaCl. Thus, with imidazole also, the swelling appears to be reversible.

contrast to the ring compounds, no hemolysis was seen with 300-mOsm. M solutions over a period of 3 hours. When allowed to stand overnight, olysis was observed in solutions at *p*H 7.8 and 7.4 but not in solutions at .0 and 6.6 nor in phosphate buffer. These results with long-standing red suspensions may not be related simply to THAM penetration since solute for example, potassium ions, could affect hemolysis time.

Effect of THAM on the Short Circuit Current of Frog Skin

sing and Zerahn[11] have shown that the amount of direct current applied duce the frog skin potential to zero is equivalent to the net inward trans- of sodium ions. In the present experiments, sulfate-Ringer's was used to mize effects of anion transport on skin potential and on short circuit cur- [15] In FIGURE 6, a typical result using phosphate-, THAM-, and imidaz- uffered media is shown. THAM apparently does not have any influence on short circuit current at the concentration studied, 22.5 mOsm., or at a fif fold greater concentration that was tested in another experiment. Imidazo on the other hand, increases both short circuit current and skin poten dramatically. Placing the imidazole-buffered solution in the chamber bathi the inside surface produced a larger change than placing this solution in outside chamber.

An obvious explanation for the difference in results with THAM and imid ole on the skin is suggested by the large differences in penetration rate in

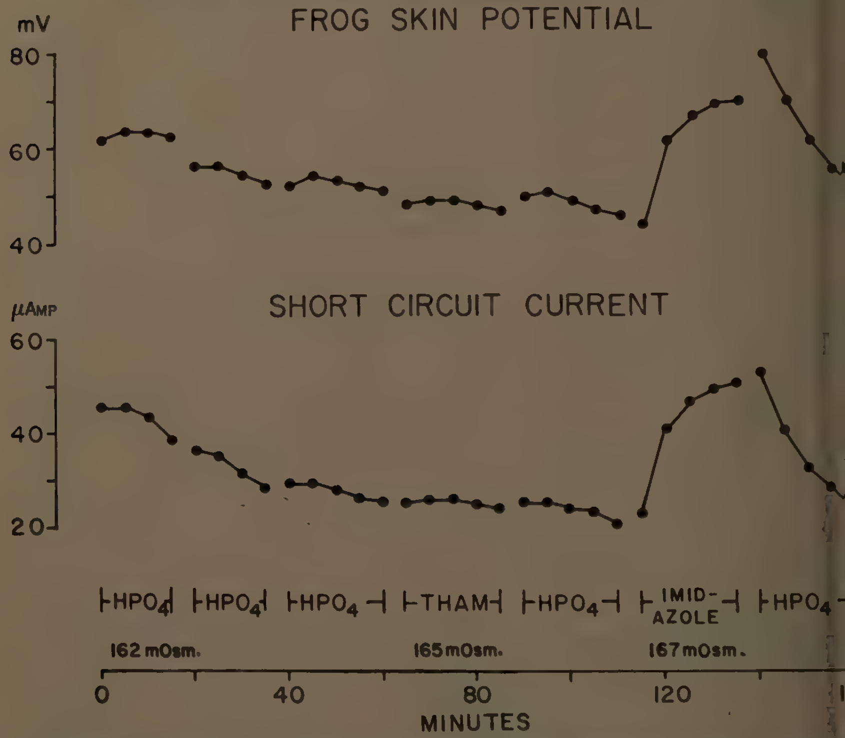


FIGURE 6. Frog skin potential and short circuit current in sulfate-Ringer's buffered w phosphate, THAM, or imidazole. All solutions had roughly the same osmolality, as sho The interrupted breaks in the curves represent washing of the chambers three times w new solution.

erythrocyte experiments. Hemolysis in imidazole took place in a matter seconds, whereas there was no hemolysis in THAM after 3 hours. The curr increase with imidazole may be due to the increase in cell *p*H following penetration of the undissociated amine since it has been shown that higher ternal *p*H is correlated with higher current output.[16] Ussing and Zerahn h also noted that a gas mixture containing 5 per cent CO_2 depresses short circ current.[11]

Concluding Remarks

A number of organic compounds have been shown to enter erythrocyt so that it was not entirely unexpected to find that organic amines also penetr

han red cell membranes. As might have been predicted, the principal
etrant appeared to be the undissociated base. A result that was not antici-
ed was the greater rate of entry of imidazole into human erythrocytes com-
ed to THAM. It was of interest to note, moreover, that the short circuit
ent in frog skin was increased by imidazole but not by THAM. The latter
ing also suggests that, following penetration, organic amines can affect
ılar processes such as active sodium transport, presumably by changing
*p*H. The hypothesis that organic amines may act as intracellular buffers
s appears to be supported. These results suggest further that the use of
ne buffers other than THAM may be of value in experimental respiratory
losis since a more rapidly penetrating amine may permit lower dosages and
e accurate control, providing undesirable side effects do not take place.

Acknowledgment

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vrence Chapman for technical assistance during various phases of this study.
ıry Tedeschi kindly offered many invaluable suggestions.

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USE OF AMINE BUFFERS IN PROTOZOAN NUTRITION*

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The toxicity for a protozoan of certain amine buffers, including tris(hydr methyl)aminomethane (Tris), is one phase of an extensive study of the r bility of certain protozoa as guides to the metabolism of higher animals. paper describes the remarkable low toxicity of Tris towards our main organism, the phagotrophic (particle-ingesting), brown-pigmented ph synthetic flagellate *Ochromonas danica.* The culture medium used was dev to permit growth at a *p*H (7.6) at least as high as that of blood. Since flagellate has exceptionally high metal requirements, supplying sufficient and trace elements in an alkaline medium seemed comparable to the prob of securing sufficient absorption of iron and other trace elements by an ach hydric athlete. Media of the type to be described may prove useful for de ing basic cytotoxic materials.

Soon after their introduction to biology as buffers for histochemical w Tris and related buffers came into general use in microbiology. A precau in its use in mammalian systems was implied by the findings of Macleod Onofrey (1954): Tris and triethanolamine competitively interfered with uptake of K^+ by *Lactobacillus arabinosus.* The advisability of fortifying K^+, Tris-buffered media for marine algae and phytoflagellates was emphas by Provasoli *et al.* (1957), who noted that at *p*H 7.5 and 8.5, 100 mg. per Tris raised the minimal requirement of the blue-green alga *Phormidium p cinum* from 0.5 mg. per cent (the minimal concentration tried) to 4 to 5 mg. cent. Similar precautions were advised for freshwater algae (Provasoli Pintner, 1960), but with some variations: the toxicity of triethanolamine counteracted by increasing the Ca concentration for *Volvox globator* and Ca + Mg for *V. tertius* and a peridinian. The toxicity of Tris for the c tomonad flagellate was removed by Mg + K. However the toxicity of 20 per cent Tris for *Volvox globator* could not be counteracted by Ca, Mg, Na or trace metals: which implied that Tris might in some organisms inte also with certain organic metabolites. Indeed the noxious blue-green *Microcystis aeruginosa* is inhibited by 0.01 *M* Tris at *p*H 8.4 (Zehnder Gorham, 1960). At this concentration, they point out, the buffering is neg ble.

Interference with K utilization might be expected to exaggerate s states. An exceptional sensitivity of *O. danica* to Tris would argue th

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it sometimes present a risk. Conversely, should Tris *not* be dangerous
hock states, this would argue that *O. danica* was an undependable guide to
nmalian uses of amine buffers. The aforementioned failure to counteract
toxicity in *Volvox* raised some chemotherapeutic possibilities: and possibly

TABLE 1*

BASAL HIGH-*p*H MEDIUM FOR *OCHROMONAS DANICA*†

ilotriacetic acid	0.02 gm.
itamic acid	0.3 gm.
ginine-L-glutamate	0.15 gm.
O_3	5.0 mg.
$O_4 \cdot 7H_2O$	5.0 mg.
paragine $\cdot H_2O$	0.08 gm.
ine	0.1 gm.
stidine $HCl \cdot H_2O$	0.28 gm.
sine HCl	0.05 gm.
lethionine	0.01 gm.
erine	0.02 gm.
$H_4)_2(SO_4)_2 \cdot 6H_2O$	8.0 mg.
in	0.5 μg.
mine HCl	0.06 mg.
e-element mixture yielding: Fe 0.66 mg., Mn 0.53 mg., 0.33 mg., Mo 0.033 mg., Cu 0.033 mg., Co 0.033 mg., 6.6 μg., V 6.6 μg., I 2.6 μg., Se 1.3 μg.	
cetate $\cdot 3H_2O$	0.05 gm.
utyrate	0.05 gm.
ylene glycol	0.5 gm.
ane-1,5-diol	0.1 gm.
glycerophosphate $\cdot 5H_2O$	0.8 gm.
ic acid (preneutralized with NaOH)	0.15 gm.
$)_4$	2.5 mg.
idrol"	0.5 gm.
(*p*H thus adjusted to 7.6–8.0).	

Nitrilotriacetic acid is the chelator, used here instead of EDTA or citrate because of the solubility of its chelates.
he high concentration of histidine means that histidine contributes appreciably to the l-buffering capacity of the medium. For as yet unclear reasons histidine smooths out gonisms and toxicities among trace elements and so most of our newer media contain r concentrations than in the past. It also may serve as a substrate.
g has been kept as low as possible because of its proneness to form precipitates.
race elements are critical. The main problem in devising this medium was that of keephem available. The need for extra Fe was most critical of all. Ca seems to have the itude of a trace element for *O. danica* and was introduced as an impurity, especially in actate.
ycerophosphate served both as buffer and phosphate-source. Inorganic PO_4 would have rise to precipitates. The usual commercial preparation was used: a mixture of the l β isomers.
Amounts/100 ml. of final medium.

hitherto unforeseen dangers. Two amino alcohols suggested themselves argets for amine buffers: (1) the 1-amino-2-propanol moiety of vitamin (2) 1-amino-2-methyl-2-propanol, a probable constituent of *Neurospora* pholipid (Ellman and Mitchell, 1954).

ne results communicated here indicate that Tris is remarkably safe to use *O. danica* grown in alkaline media. Nevertheless as some related comds proved remarkably toxic we think it worth while to communicate these ts as a matter of chemotherapeutic interest even though we have not identified their cellular targets. Finally, some miscellaneous uses of a buffers in protozoan nutrition will be mentioned.

Results

References to the physiology of *Ochromonas danica* and to the techn used in this study are given by Aaronson and Baker (1959). Further d are given by Hutner *et al.* (1958).

The high-*p*H medium developed for comparisons of the toxicity of a alcohols is shown in TABLE 1. One of several such experiments is sho TABLE 2. We think it premature at this time to present the details o very limited counteraction of the toxicities noted by excess K, choline, 1-amino-2-propanol, alone and in combination, as these were active only very limited concentration range: apparently something else is interfered as discussed in the introduction of this paper; indeed, this topic is outsid main theme of this monograph. What does emerge clearly is the innoc ness of Quadrol, and the almost complete harmlessness of Tris and trieth amine.

TABLE 2

TOXICITY OF AMINO ALCOHOLS FOR *OCHROMONAS DANICA* IN HIGH-*p*H MEDIU

2-Methyl-2-amino-1-propanol	0.1
2-Methyl-2-amino-1,3-propanediol	0.25
1-Dimethyl-1-amino-2-propanol	0.15
1,3-Diamino-2-propanol	0.02
1-Amino-2-propanol	0.1
Tris	0.5
Dimethyl ethanolamine	0.25
2-Amino-2-ethyl-1,3-propanediol	0.04
3-Amino-1-propanol	0.15
Triethanolamine	0.5
Quadrol	1.5

* Values refer to concentration per cent reducing growth to 50 per cent of control

Because of the uncertainties surrounding the basal medium, intensive studies of rev factors have been put off temporarily. It is conceivable that some of these amino al may be inhibitory through their effectiveness in sequestering such essential trace ele as Cu and Zu. Since these ions are tightly bound by certain polyamines, such comp as ethylenediamine should be used as controls, and the tolerances for trace elements c defined. In recent experiments we have found, too, striking interactions between the of substrate and the sensitivity of the organism to imbalances in respect to amino ac the leucine-isoleucine-valine-phenylalanine-tryptophan-methionine constellation; these acids are notorious for their proneness to go out of balance for organisms ranging fron teria to man.

The *p*Hs of the stock solutions of the amines were carefully adjusted with succinic a the *p*H of the basal medium.

Tris (0.2 per cent) has been used successfully in media for *Tetrahy pyriformis* (Baker *et al.*, 1960).

Summary and Conclusions

Amine buffers, for example, Tris and triethanolamine, and dibasic a alcohols such as Quadrol, are remarkably noninhibitory for the chrysom flagellate *Ochromonas danica* in a high-*p*H medium, even in media conta minimal potassium.

brief review of the microbiological literature indicates that Tris may ice K or Ca deficiencies, or in some algae, be inhibitory by some unknown hanism. The low toxicity towards *O. danica*, an organism thought to have exceptionally metazoanlike metabolism, may be construed as supporting reliability of *O. danica* as a guide to the cellular metabolism of higher nals.

ses of triethanolamine as a solvent for hemin and of Quadrol as an all-pose, safe alkali for alkalinizing culture media, are mentioned.

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ADDENDUM

Miscellaneous Microbiological Uses of Amine Buffers

eme solvent. Some years ago we needed stable solutions of hemin, an essential growth r for most trypanosomid flagellates, since aqueous alkaline solutions gave rise to pre-ates or irregular growth. The difficulty presumably arose from dimerization and oxida- Caffeine in excess formed a stable hemochromogen (Keilin, 1943). Caffeine-hemin ions did not prove to be the answer, for the concentration of caffeine needed was close to ation and to inhibitory concentrations. Simple solutions of hemin in 50 per cent (w/v) ous triethanolamine, 5 mg./ml., proved entirely satisfactory (Cowperthwaite *et al.*, 1953) became the standard method in other laboratories as well. In many unpublished experi-s solutions made up similarly in 50 per cent Quadrol have proved equally satisfactory; d it is likely that, thanks to its high viscosity, Quadrol slows dimerization and hinders the tration of oxygen more. We know of no published studies on complex formation between e alcohols and porphyrins.

uadrol as All-Purpose Alkali.* The *p*H of a 0.1 per cent Quadrol solution is 9.8, which it above the buffer range of almost all culture media. The growth of microbes in very ine media has received little attention, but when this subject does develop, Quadrol should useful buffer. (Almost nothing is known of the mode of action of biological polyamines, these penetrate best where their ionization is minimal, that is, in very alkaline media, h makes desirable the development of such media.)

uite apart from their buffer action, we find Tris, triethanolamine, and Quadrol much convenient in neutralizing culture media than NaOH or KOH. Complicated culture a are conveniently stored as "dry mixes." For the sake of ready solution, these mixes

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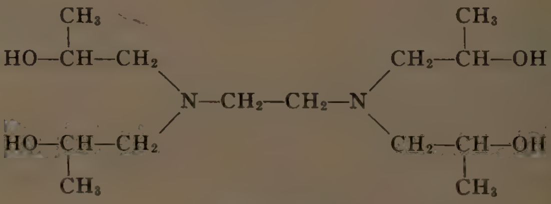
See footnote, TABLE 1.

are designed to yield a solution of pH 6.0 or lower. The stability of Tris is such that the amount of Tris is determined for the desired alkalinization of the basal medium, the amount will serve for the next preparation: in effect, Tris permits a "double dry-mix" nique. Media unlikely to be made again are conveniently adjusted directly with 50 pe Quadrol. Quadrol solutions are stored in large glass-stoppered bottles. The Quadro as a lubricant: the stoppers never freeze. The entrance of CO_2 is thus hindered and the tions remain at practically constant strength. This low uptake of CO_2 results in med becoming more alkaline upon autoclaving because of bicarbonate decomposition. Quadrol has two equivalents of alkali, it has advantages when it is important that the m have as low an osmotic pressure as possible. The high viscosity of Quadrol solutions su its use as an alkaline glycerol-like compound preservation of spermatozoa and red ce freezing. Since histamine liberators are usually strongly basic or highly surface- (Miles and Wilhelm, 1960), Quadrol may present a hazard when included in parenteral rations, which suggests the new problem of whether such toxicity is controllable with histamines. Quadrol is irritating but not corrosive to mucous membranes and so its u stead of NaOH or KOH contributes to laboratory safety.

The complicated array of substrates was assembled as a replacement of carbohy Carbohydrate-containing media tend to become acid, and this reduced the toxicity of so the amine alcohols being studied, and so interfered with counteraction-of-toxicity st "Quadrol" is *N,N,N′,N′*-tetra-kis(2-hydroxypropyl)ethylenediamine (Wyandotte Che Corp., Wyandotte, Mich.).



QUADROL

K_2SO_4 supplies the minimum K supporting full growth. K was kept low in order t plify detection of K antagonisms.

This medium may be contrasted with that for pH 5.0 described by Aaronson and (1959).

III. Effects of Amine Buffers on CO_2 Storage and Acid-Base Balance

THE EFFECT OF 2-AMINO-2-HYDROXYMETHYL-1,3-PROPANEDIOL ON BLOOD-BUFFERING CAPACITY

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The Buffer Capacity Concept

uffer value, from which buffer capacity is derived, was defined by Van e (1922) as the differential ratio $d\mathrm{B}/dp\mathrm{H}$, where $d\mathrm{B}$ is the increment of ng base (in gram equivalents) added to 1 l. of buffer solution, and $dp\mathrm{H}$ is the ltant increment of $p\mathrm{H}$. Increments of strong base (in modern nomencla- , OH^-) or strong acid (H^+) are equivalent to negative increments of strong or strong base, respectively. When measurable increments are indicated , buffer value can be denoted in the following ways:

$$\frac{d\mathrm{B}}{dp\mathrm{H}},\ \frac{d\mathrm{OH}^-}{dp\mathrm{H}},\ \frac{-d\mathrm{H}^+}{dp\mathrm{H}},\quad \text{or}\quad \frac{\Delta\mathrm{OH}^-}{\Delta p\mathrm{H}},\ \frac{-\Delta\mathrm{H}^+}{\Delta p\mathrm{H}}.$$

he dimension is: equivalent $\times$ liter^{-1}.

uffer capacity is similar to buffer value, but the amount of buffer is arbitrary ume or mass). Buffer capacity is thus an extensive quantity. However, er capacity is often used in the sense "per liter capacity", that is, synony- s with buffer value. Buffer capacity can be denoted as the same ratios as er value, but the dimension is: equivalent.

uffer capacity is the slope of a titration curve, where $p\mathrm{H}$ is plotted against d base. The actual value depends on the amount and composition of the er system, and its actual state (temperature), and hence on the actual pH.

uffer capacity is an additive quantity when the buffer systems to be mixed referred to the same $p\mathrm{H}$. No chemical reactions must take place, and ity factors must be unchanged. If measurable increments ($\Delta p\mathrm{H}$) are idered these must be equal.

Buffer Capacity of Blood

its general form the buffer capacity concept can be used also for blood. ever, the results are without physiological importance. This is due to the ue properties of carbonic acid (carbon dioxide) which is produced in large ınts, and regulated to a certain carbon dioxide tension ($p\mathrm{CO}_2$).

r the use of blood, the buffer capacity should be restricted to be measured onstant $p\mathrm{CO}_2$. This limitation has no influence on the additive property, ne point of practical interest should be mentioned. Generally, some water buffer capacity zero) can be added freely during the titration without ence on $p\mathrm{H}$. However, when $p\mathrm{CO}_2$ is kept constant, such water will appear solution of carbonic acid with a measurable buffer capacity.

e limited buffer capacity (denoted as $[d\mathrm{OH}^-/dp\mathrm{H}]_{p\mathrm{CO}_2}$) can be supple- ed with the ratio $dp\mathrm{CO}_2/dp\mathrm{H}$, and, for the sake of completeness, with $/dp\mathrm{H}$ to describe the buffer action of the system.

e ratios $(d\mathrm{OH}^-/dp\mathrm{H})_{p\mathrm{CO}_2}$ and $dp\mathrm{CO}_2/dp\mathrm{H}$ of blood can be illustrated by

means of a nomogram, described in detail elsewhere (O. Siggaard Ande
and K. Engel, 1960). The nomogram, as shown in FIGURE 1, is based on
fact that within physiological ranges the variation of log pCO_2 with p

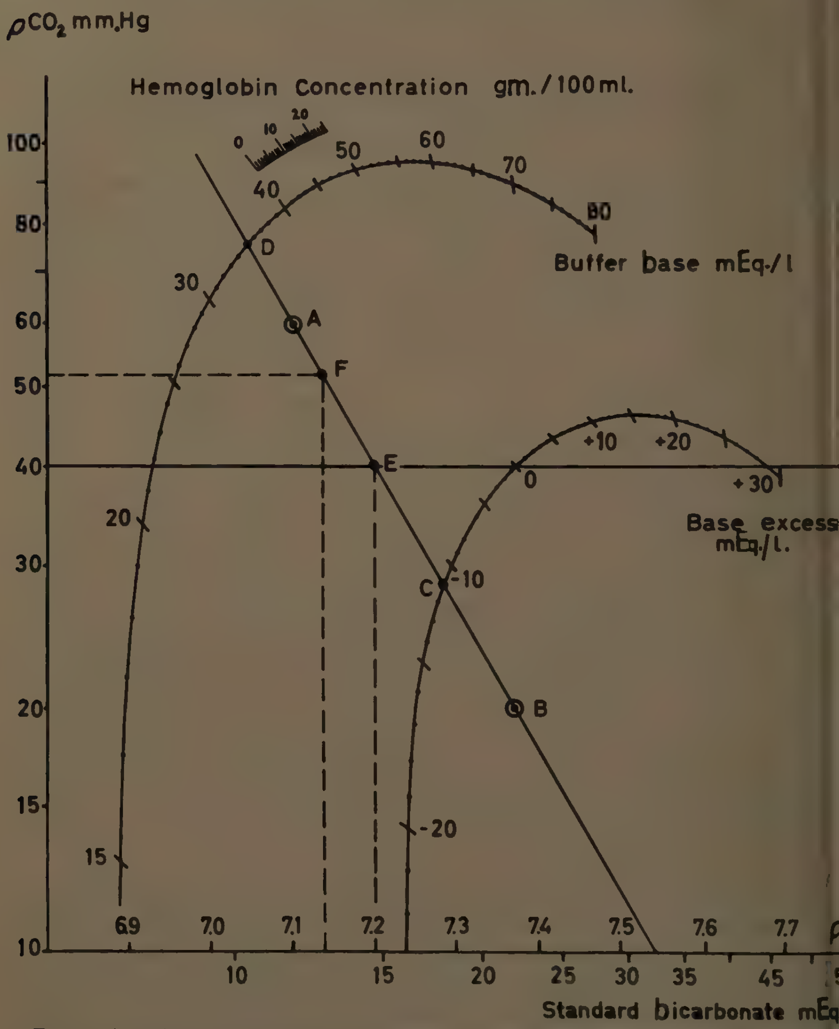


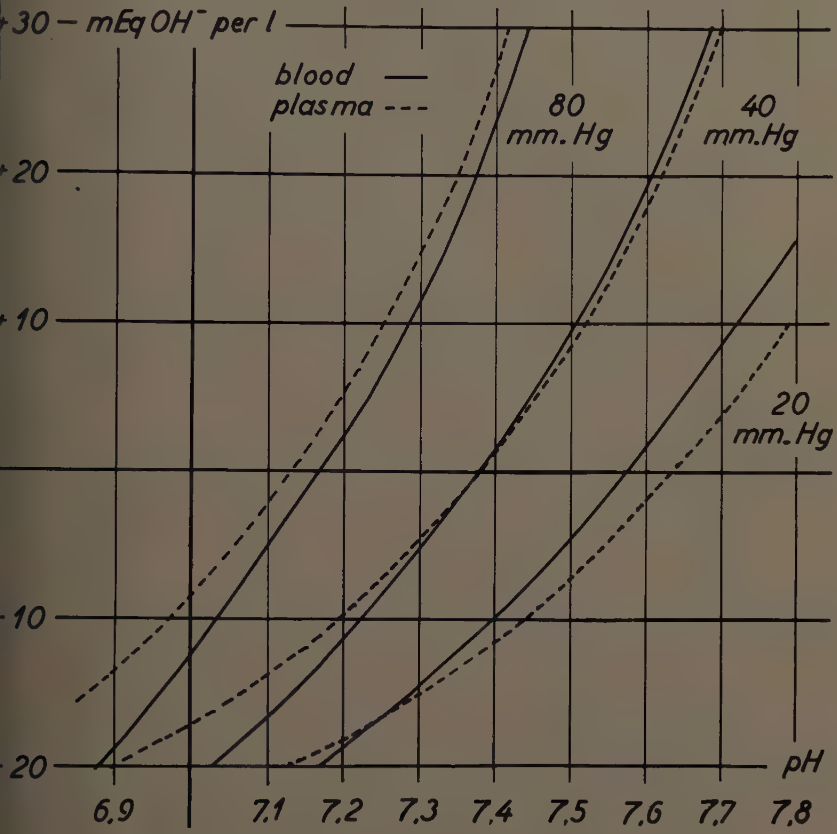
FIGURE 1. The nomogram used to calculate the acid-base status of blood. Points A
B are the measured ones. The straight line through these points is displaced to the left
the normal position, indicating the presence of a nonrespiratory acidosis (base excess
mEq/l.).

practically rectilinear for any blood sample. The intersection of this line
a pH-axis at a certain pCO_2 level (for example, 40 mm. Hg) is moved by addi
of strong acid or base. The nomogram allows corrections for varying he
globin concentrations and, eventually, oxygen saturation.

For the practical use of the nomogram the sample is equilibrated with
different CO_2 tensions (and oxygenated), and the corresponding pH values

d. All can be done on a micro scale with the method described elsewhere
Siggaard Andersen *et al.*, 1960).

The nomogram enables one to read any quantity relevant for characterizing
acid-base status of the blood. Two quantities, base excess and standard
arbonate, describing the nonrespiratory status (Astrup *et al.*, 1960) will be
ntioned here. Base excess is the amount of OH^- (in mEq/ l.) which has



IGURE 2. Titration curves of normal blood (15 gm. hemoglobin per 100 ml.) and plasma,
ined at fixed carbon dioxide tensions, at 38° C. When $p\text{CO}_2$ is kept at 40 mm. Hg normal
d and plasma has $p\text{H} = 7.38$.

n added to the sample to bring its log $p\text{CO}_2/p\text{H}$ line to its actual place from
normal place. The normal place (base excess zero) is through the point
$\text{O}_2 = 40$ mm. Hg), ($p\text{H} = 7.38$). The intersection with the pH axis at
$)_2 = 40$ can also be expressed as the "standard bicarbonate" value, cal-
ted from the Henderson-Hasselbalch equation as $p\text{H}_{40} = 6.10 + \log$ (stand.
$/0.03 \times 40$). The normal value is 22.8 mEq/l., that is, the bicarbonate
centration of normal plasma.

rom the nomogram the titration curves for blood with various hemoglobin
centrations can be derived for any $p\text{CO}_2$ wanted. Such curves are shown
IGURE 2.

The Buffer Capacity of 2-Amino-2-hydroxymethyl-1,3-propanediol

FIGURE 3 illustrates the buffer capacity of 2-amino-2-hydroxymethyl-1 propanediol (= THAM*) about pH 7.38. The curves for 100 mmole 200 mmole illustrate the additive property of the buffer capacity, as the o nates of the latter are twice the values of the former. It appears that a TH

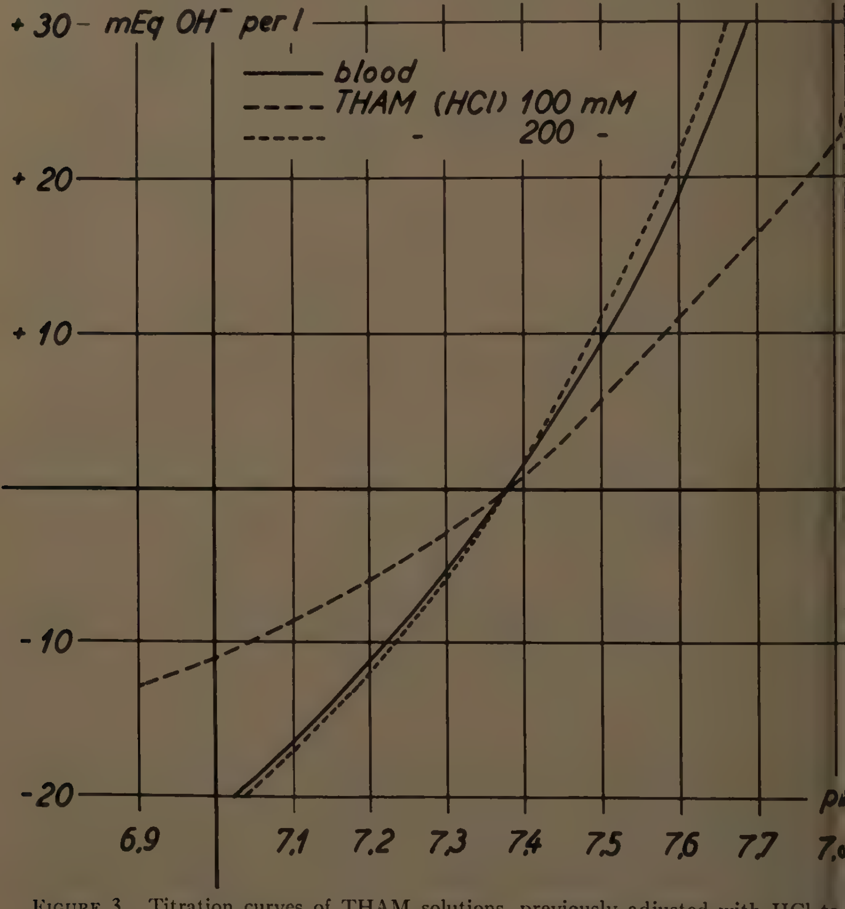


FIGURE 3. Titration curves of THAM solutions, previously adjusted with HCl to 7.38 at 38° C. No CO_2 was present. For comparison the titration curve of normal bloo pCO_2 = 40 mm. Hg is shown.

solution of about 170 mmole (THAM + THAM, HCl) has about the sa buffer capacity as normal blood, this being titrated at pCO_2 40 mm. Hg. other words, to double the buffer capacity of normal blood requires 170 mm of THAM + THAM, HCl (previously adjusted to the pH of blood) to be solved in each liter of blood.

It is interesting to note that a 170-mmole solution of THAM at pH 7.38 ha total molar concentration ("osmolarity") of 300 mmole, which is calculated ıns of the titration curve of THAM, shown in a different form in FIGURE 4. ıs to double the buffer capacity of normal blood with THAM would require ıbling of the osmotic concentration.

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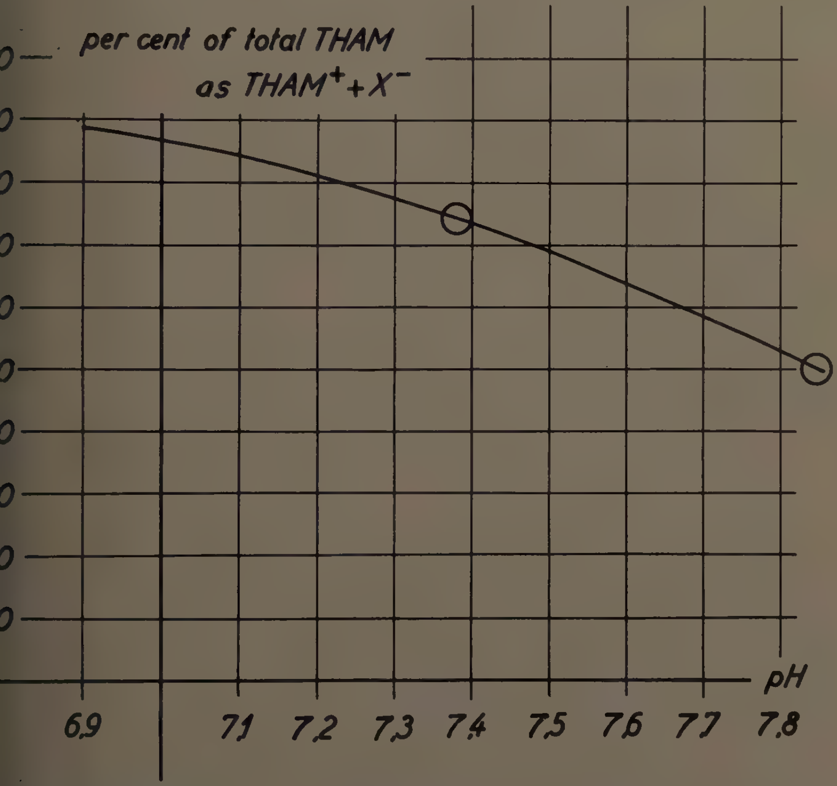
* THAM for tris(hydroxymethyl)aminomethane.

Experimental Buffer Capacities of Blood-THAM Mixtures

n a series of experiments THAM + THAM, HCl, previously adjusted to e *p*H 7.38 when dissolved, was added to blood to yield concentrations of al THAM ranging from 10 to 50 mmole/l. blood; these were considered



IGURE 4. Titration curve of THAM solutions, showing the fraction of total THAM rring in the dissociated form at various *p*H values.

onable values from a physiological point of view. Known amounts of H^+ OH^- were added.* The samples were then equilibrated with 2 known tensions (about 30 and 60 mm. Hg) and the *p*H values were read (Andersen l., 1960). A rectilinear log *p*CO /*p*H curve was drawn on the nomogram IGURE 1 and titration curves analogous to those in FIGURES 2 and 3 were wn. (It should be remembered, that the nomogram of FIGURE 1 can not be l directly when appreciable amounts of THAM are present in the blood. ndard bicarbonate can be read directly, but the buffer base and base excess es must be altered.)

See under *Hemolysis in Experiments with THAM.*

The equilibrations and pH measurements have been performed within o half to three hours after addition of THAM and acid or base. No attenti has been paid to the distribution of THAM between plasma and erythrocyt As H^+ and OH^- readily penetrates the erythrocyte membrane, and the eryt rocyte readily changes its volume, no trouble can be expected in experimen with blood due to an abnormal equilibrium between intra- and extracellu pH.

The results obtained were consistent with those calculated by addition of t curves for pure blood and for the respective concentrations of THAM.

Neutralizing Blood with THAM

It might be of interest to neutralize an acid blood sample (to pH 7.38 pCO_2 40 mm. Hg) by means of the weak base THAM. The amount of THA necessary per liter of blood can be calculated as follows. Base excess of t blood is first determined (in case of acid blood the value is negative). T equivalent amount of THAM is used, acting as OH^-, according to THAM $H_2O \rightarrow THAM^+, H + OH^-$. A further amount of THAM is used unreact to fulfill the requirement that at pH 7.38 only 74 per cent of total THAM is the salt form (FIGURE 4). One then has

$$\frac{\text{THAM}^+, \text{H}}{\text{THAM}^+, \text{H} + \text{THAM}} = 0.74 = \frac{\text{base excess}}{\text{THAM}^+, \text{H} + \text{THAM}},$$

or

$$\text{THAM to be added, equal parts per liter} = \frac{\text{base excess}}{0.74}.$$

If THAM is added as solution, a correction for solvent water is requir As pCO_2 is kept constant at 40 mm. Hg, this water should have a bicarbon concentration of 22.8 mmole. This can be done by adding THAM to yie bicarbonate according to $THAM + CO_2 + H_2O \rightarrow THAM^+, H + HCO$ plus the unreacted THAM to fulfill the requirement mentioned above.

Neutralization of alkaline blood with THAM, HCl would be unreasonable THAM, H^+ is too weak an acid.

Blood, THAM Solutions, and Their Mixtures*

For blood the relationship between pCO_2 and pH can be found from t nomogram (FIGURE 1).

For THAM solutions of various concentrations of total THAM and with a initial pH and pCO_2 the relationship can be calculated by the equations

$$p\text{H} = 6.10 + \log \frac{\text{HCO}_3^-}{0.03 \times p\text{CO}_2}, \qquad p\text{H} = 7.82 + \log \frac{\text{THAM}}{\text{THAM}^+, \text{H}},$$

and

$$\frac{\text{THAM}^+, \text{H} \times \text{HCO}_3^-}{\text{THAM} \times 0.03 \times p\text{CO}_2} = \frac{10^{-6.10}}{10^{-7.82}}.$$

* In the ratio $dp\text{CO}_2/dp\text{H}$.

$p\mathrm{CO}_2$ is changed to $p\mathrm{CO}_2'$ new equivalents of THAM^+, H and HCO_3^- are rmed at the expense of THAM. Therefore one may write:

$$\frac{(\mathrm{THAM}^+, \mathrm{H} + \mathrm{X}) \times (\mathrm{HCO}_3^- + \mathrm{X})}{(\mathrm{THAM} - \mathrm{X}) \times 0.03 \times p\mathrm{CO}_2'} = \frac{10^{-6.10}}{10^{-7.82}}.$$

hen X is found, the new pH is easily calculated.

However, the ratio $dp\mathrm{CO}_2/dp\mathrm{H}$ has no additive properties. FIGURE 5 shows CO$_2$, pH lines of THAM solutions, calculated as above. It appears that a 0 mmole solution of THAM (+ bicarbonate, passing through (pH = 7.38), CO$_2$ = 40 mm. Hg) behaves nearly as normal blood, but its line is definitely rved. When the lines for pure bicarbonate solution, and 100 and 200 mmole HAM solutions are compared, it is apparent that the effect of THAM on the pe of the line is decreasing with growing THAM concentrations.

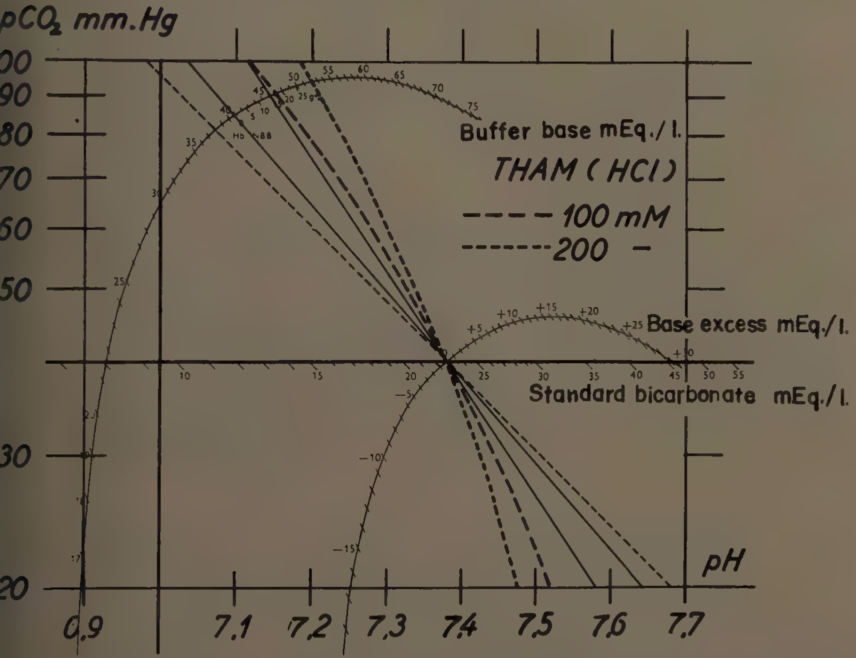


FIGURE 5. Thick-lined curves show the $p\mathrm{CO}_2$, pH-relationship calculated for THAM utions, adjusted with bicarbonate and HCl to pH 7.38 at $p\mathrm{CO}_2$ = 40 mm. Hg (at 38° C). in-lined curves show the relationship of pure bicarbonate solution (dotted line), normal sma and normal blood (15 gm. hemoglobin per 100 ml.). The graph is essentially the mogram of FIGURE 1.

The $p\mathrm{CO}_2$, pH lines, actually measured on blood with concentrations of total HAM ranging from 10 to 50 mmole showed a marked effect of THAM on e steepness of the lines. Thus normal blood (15 gm. hemoglobin per 100 ml.) th 50 mmole THAM had a slope nearly like that of blood with 25 gm. hemobin per 100 ml. Due to the failure of additive properties of $dp\mathrm{CO}_2/p\mathrm{H}$ no tempt was made to predict the course of these curves.

*Blood**

Unlike the fixed acids and bases, the amounts of CO_2 added to the bl in the organism is so immense when compared with the CO_2 content the blood that the addition can take place only if an excretion of CO_2 is go on with the same rate. Otherwise, pCO_2 would rapidly exceed the physiologi limits. Further, the normal CO_2 transport of blood is based on a special action: oxyhemoglobin ↔ hemoglobin. Therefore the problem of CO_2 cap ities of blood has been without the scope of the present *in vitro* investigati

Supplementary Experiments

As it was found that the titration curves of blood-THAM mixtures beha in the predicted manner, it was suggested that THAM, when added to blo behaves just as does a weak base without chemical side reactions. The pr lem of activity coefficients, however, could not be resolved by these experime due to experimental error.

In a series of experiments 1 volume of 0.3 *M* THAM, adjusted to *p*H 7.38, added to 5 to 20 volumes of water, 25 mmole bicarbonate solution, plasma fr which the CO_2-bicarbonate system had been removed,† and normal blo respectively, all solutions previously adjusted to *p*H 7.38. The mixing made anaerobically at 38° C. The mixtures always got a slightly lower the decreases being about 0.01 to 0.02 *p*H units except for water where the d was about 0.03 *p*H units. The results can be ascribed to a dilution effect on THAM solution, and there should be no reason to change *p*K value (6.10) carbonic acid in plasma when THAM is added in concentrations below mmole.

Hemolysis in Experiments with THAM

In most experiments THAM was added to blood in the following way: proper amount of a THAM solution, for example 500 mmole, adjusted to 7.4 at 38° C. with hydrochloric acid, was dried at low temperature in a sm test tube. Two ml. of blood was added and immediately rotated. The fi concentrations of THAM were from 10 to 50 mmole. In all cases a sli hemolysis was noted after the dissolution of THAM.

In some experiments 1 volume of 0.3 *M* THAM solution (*p*H 7.4) was ad to 10 volumes of human whole blood, which was efficiently stirred. In s cases a slight hemolysis was also noted.

It was found that the corresponding amounts of THAM could be dissol without causing hemolysis if the THAM was first dissolved in the plasma the erythrocytes added afterwards.

The sodium hydroxide and hydrochloric acid added for titration was alw added in the following way: 2.00 ml. of blood (with or without THAM) centrifuged. Fifty μl. of NaOH or HCl (or water) with various concentrati (1, 0.8, 0.6, 0.4, and 0.2 *N*) were added and mixed with the supernatant plas before remixing with the red cells. If the acid or base was added direc to whole blood, even with stirring, hemolysis was observed.

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* In the ratio dCO_2/dpH.

† 6 *M* HCl was added until *p*H was below 4. CO_2 was expelled *in vacuo*, and *p*H adju to 7.38 with 2 *M* NaOH.

Conclusion

2-Amino-2-hydroxymethyl-1,3-propanediol (THAM) is a weak base, which—
the blood—behaves exactly as can be predicted from the properties of blood
d from the properties of THAM as a weak base.

There is no possibility to *stabilize* the blood *p*H significantly, neither against
ed acid or base, nor against CO_2, by merely increasing the *buffer action* of
od by addition of THAM + THAM, HCl, unless blood *p*H is already near
physiological limits.

As a base, THAM is strong enough to be usable for correction of nonrespir-
ry acidosis.

Respiratory acidosis should generally be treated by artificial ventilation. If,
some acute cases, a chemical treatment is preferred, THAM might be pref-
ble to sodium hydroxide or sodium carbonate, as the injected solution would
less caustic.

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CIRCULATORY CHANGES FOLLOWING RAPID CORRECTION SEVERE HYPERCAPNIC ACIDOSIS BY 2-AMINO-2-HYDROXYMETHYL-1,3-PROPANEDIOL*

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The discovery that 2-amino-2-hydroxymethyl-1,3-propanediol (THA can be given *in vivo* to titrate carbonic acid, thus correcting a respirat acidosis,[1] offers an avenue for the further investigation of many of the phenc ena associated with hypercapnia. By the use of THAM it is possible to incre the *p*H of biological fluid despite the simultaneous increase of its P_{CO_2} carbon dioxide content as the result of a carbon dioxide load.

This potential offers an opportunity to examine in another way the phenc enon of posthypercapnic ventricular fibrillation,[2] the mechanism of which mains obscure despite numerous studies[3,4,5] directed toward its understandi An investigation was designed to observe some of the circulatory, electrocarc graphic, and electrolyte changes during the rapid correction by THAM of acidosis produced by prolonged hypercapnia.

Methods

Randomly selected mongrel dogs were anesthetized with 30 mg./kg. sodium pentobarbital, intubated, and respired with oxygen using a Starl pump. Succinylcholine chloride in 10 mg. dosage was employed in m studies to insure immobility and to allow artificial respiration to be acco plished easily. This compound was repeated as necessary to a maximum tc of 30 mg. The femoral artery on one side was cannulated for registration arterial blood pressure by Statham strain gauge and Grass recorder, while on other side a Cournand needle was inserted for sampling arterial blood. Lea of the electrocardiogram was recorded.

Following control determinations, the breathing mixture was changed to per cent carbon dioxide in oxygen which was administered for the next 2 hou After additional blood sampling and ECG and blood pressure recording, mixture was again changed to 40 per cent carbon dioxide in oxygen for subsequent 2 hours. After sampling once again, intravenous infusion o molar THAM in distilled water was begun at a rate calculated to prod approximate correction of the acidosis at the end of a 5-min. interval. T was accomplished by the administration of approximately 2.5 ml./kg. mEq/kg.) during the first minute and 1.25 ml./kg. (2.5 mEq/kg.) for each the subsequent 4 min. During the administration of THAM, electrocarc graph and blood pressure recordings were made continuously. Arterial bl was sampled 1, 2, and 5 min. subsequent to the start of the infusion. I studies the electrocardiogram was then observed until asystole terminated eriment. In 4 additional studies, 1 gm. of calcium chloride was adminis- d intravenously following the 5-min. specimen as asystole was impending. additional specimen of arterial blood was then taken 1 min. subsequent he infusion of calcium.

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lood samples were collected from the femoral artery needle anaerobically yringes greased with stopcock grease and, after *p*H determination, were trifuged immediately at 2500 rpm for 5 min. The dead space of the syringe filled with a drop of mercury and a small amount of heparin powder dis- ed in distilled water. Blood *p*H was determined with a Radiometer *p*H er using a Sanz or Astrup glass capillary electrode surrounded by a tem- ature-controlled water jacket maintained at 37° C.

he plasma total carbon dioxide content was determined in the Kopp-Natel- microgasometer, using the method of Holaday and Verosky.[6] The arterial on dioxide tension was calculated using the Henderson-Hasselbach equa- , assuming *p*K of 6.10.

lasma sodium and potassium ion concentrations were determined on aliquots en with a Sanz pipette using an internal standard Patwin flame photometer.[7] sma chloride was determined coulometrically using the Cotlove chlorido- er.[8] Glucose was determined enzymatically with a coupled glucose oxidase em.[9] Calcium was determined in the last three studies in which calcium oride was administered, using a modification of the murexide titration.[10] sma osmotic pressure was determined in four studies by freezing point ression, using the Fiske osmometer. The specimens for this determination e collected rapidly in open test tubes and centrifuged immediately. Oxygen rations were determined in seven experiments using the spectrophotometric hod of Nahas[11] as modified by Holling.[12]

Results and Discussion

lectrocardiogram. The electrocardiographic changes are typified by FIGURE d, during the 4 hours of CO_2 inhalation they were usually minimal. Usu- , but not always, there was some cardioacceleration and depression of the segment in lead 2. The rhythm remained of sinus origin without atrio- tricular or intraventricular block.

ollowing the administration of THAM, several characteristic alterations eared. Atrioventricular block developed and progressed to complete oventricular dissociation and, finally, to sinus arrest. The downward lacement of the pacemaker resulted in nodal rhythm progressing to idio- tricular rhythm. There was sagging and slurring of the S-T segments and, he abnormality progressed, a widening QRS complex was followed by a ly coved S-T segment and tall, peaked T waves. The electrical activity he heart gradually became less frequent and of lower amplitude until tole, unless it occurred abruptly at an earlier stage. Ventricular tachy- ia or fibrillation was not seen.

the four animals given calcium chloride, the drug was administered when electrocardiographic changes described above were well developed and st of the heart seemed imminent. In each of these cases after a brief y for circulation of the drug, the progression of electrocardiographic dete- ation was reversed and, after a greater or lesser time, the complexes returned to the wave form prior to the administration of THAM. Each of these anim survived the experiment and were later sacrificed. A typical electrocard graphic record from one of these animals is presented in FIGURE 2.

The arterial blood pressure was well maintained while the animal was brea ing carbon dioxide. With the administration of THAM and the developme

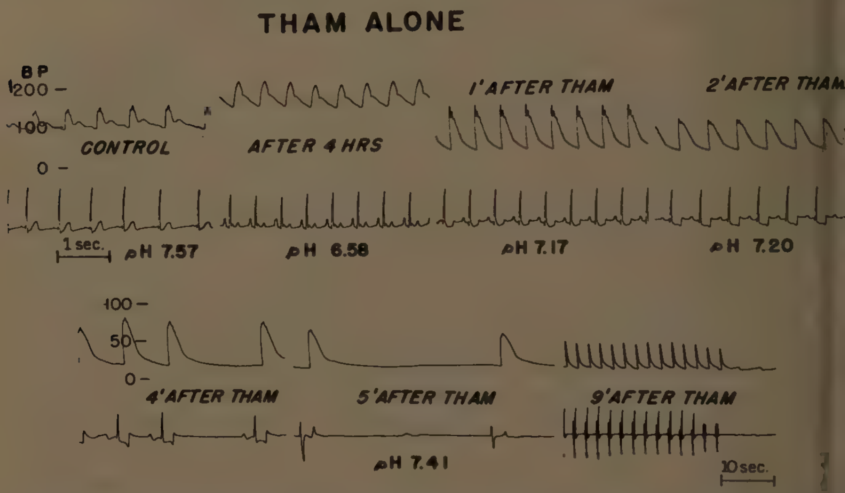

FIGURE 1. Effect of THAM on blood pressure (*above*) and ECG after 4 hours of hy capnia. Note change in sensitivity of strain gauge recorder on second line. Descriptio text.

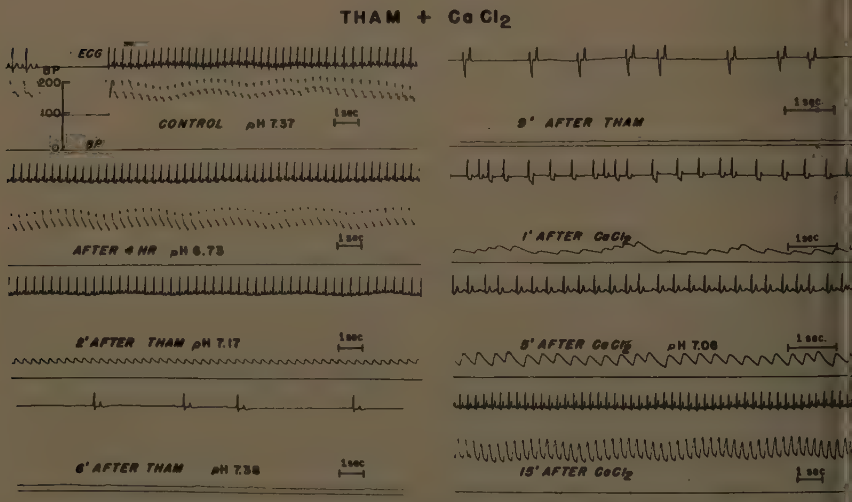

FIGURE 2. Effect of calcium chloride on ECG (above) and blood pressure in THA treated hypercapnia. Sequence from above downward. One gm. of calcium chloride ministered at the end of the strip marked “9′ after THAM.” Description in text.

he electrical disturbances in the heart muscle, the blood pressure fell rapidly, sumably because of a concomitant reduction in the contractility of the ocardium (FIGURE 1). The mechanical systole of the heart in many cases med almost to have disappeared prior to the electric arrest. In each case en calcium chloride was given intravenously, however, the resumption of ctrical activity and the parallel improvement in the mechanical force and od pressure occurred promptly.

lasma *p*H, P_{CO_2}, and bicarbonate concentrations are reported in TABLE 1. should be noted that the "mean *p*H" is conventional and does not in fact resent the mean hydrogen ion concentration. The severe *p*H changes are sistent with values previously reported in this type of study. The striking nge that has not been seen previously is the extremely rapid increase in sma bicarbonate following the administration of the buffer. The source of

TABLE 1
EFFECTS OF THAM ON HYPERCAPNEMIA

Dog No.	Control			CO₂ 4 hour			THAM			Calcium		
	*p*H	P_{CO_2} mm. Hg	HCO_3^- mEq/l.	*p*H	P_{CO_2} mm. Hg	HCO_3^- mEq/l.	*p*H	P_{CO_2} mm. Hg	HCO_3^- mEq/l.	*p*H	P_{CO_2} mm. Hg	HCO_3^- mEq/l.
1	7.57	23	20.1	6.58	358	32.3	7.41	144	88.3			
2	7.41	34	20.7	6.71	292	35.7	7.38	145	82.4			
3	7.35	42	22.8	6.67	321	35.7	7.13	198	64.6			
4	7.21	61	23.9	6.63	351	35.8	7.09	234	68.0			
5	7.38	35	19.9	6.62	319	31.7	7.39	171	100.2			
6	7.38	41	23.2	6.70	362	33.0	7.37	167	92.4			
7	7.37	41	23.0	6.73	278	35.6	7.38	147	84.8			
8	7.52	23	18.1	6.79	224	33.0	7.36	133	72.1	7.25	180	75.8
9	7.42	39	24.5	6.63	374	37.7	7.16	260	90.2	7.14	267	87.2
10	7.32	39	19.5	6.58	330	30.0	7.28	158	71.3	7.03	248	64.0
Mean	7.39	38	21.6	6.66	321	34.1	7.30	176	81.4			

s bicarbonate is necessarily the intracellular carbon dioxide store accumued during the four hours of elevated inspiratory CO_2. The abrupt lowering the extracellular hydrogen-ion concentration establishes a large intra- to racellular diffusion gradient for CO_2 as the extra-cellular H_2CO_3, hence $_2$, falls. This change also implies that the penetration of the cell membrane THAM is relatively slower than by CO_2, a conclusion implicit in other k[13] on the volume of distribution of THAM. The net result of the CO_2 t, however, is that correction of the intracellular acidosis does not necessarily far behind that of the extracellular acidosis despite the slow intracellular vement of the buffer.

lasma osmolarity in a limited number of experiments is reported in TABLE A small rise is seen with the administration of carbon dioxide and a further ll rise with the buffer. Assuming a plasma volume equal to 5 per cent of body weight, the addition of 15 mEq of 2 molar material per kilogram to plasma compartment might be expected to double the plasma osmolarity.

The much smaller increase of approximately 11 per cent seen in infusion this hypertonic material may be due in part to entry of water into the plasm as is suggested by the dilution of plasma sodium, chloride, and glucose (s below). In addition, early movement of sodium ion out of plasma may occu in response to a Donnan effect resulting from the relatively slow transfer the highly concentrated THAM. Finally the binding of THAM to plasm protein (M. H. Holmdahl and G. G. Nahas, unpublished observations) w reduce its osmotic activity. The relative contribution of these effects to th observed changes can not be evaluated from the data available, but it seem unlikely that they can be explained by dilution alone.

Plasma electrolytes and glucose concentrations are reported in TABLE The sodium concentration is unchanged during hypercapnia. Correction the acidosis with buffer produces a rapid fall in plasma sodium concentratio and smaller changes in chloride and glucose as discussed above. The increa in plasma potassium concentration during hypercapnia is similar to that r ported previously.[3] The further increase in potassium occurring during cc

TABLE 2

EFFECTS OF THAM ON PLASMA OSMOLARITY* DURING HYPERCAPNIA

Dog No.	Control	CO_2 4 hour	THAM	Calcium
7	302	325	379	—
8	238	296	313	309
9	293	325	365	358
10	297	329	361	396
Mean	283	319	355	354

* mOsm/l.

rection of the acidosis has also been reported during correction of hypercapn acidosis by CO_2 washout.[3,13] It should be pointed out also that the increa in potassium occurs despite the presumed plasma dilution reflected in the oth changes reported in TABLE 3.

Total calcium concentration is reported in TABLE 4 for a limited number studies and is essentially unchanged during hypercapnia, but falls sharp with the correction of the acidosis. This fall occurs at a time when the risi pH undoubtedly produces a depression of the ionized fraction of plasma ca cium as well.[14] Infusion of calcium produced a rise in plasma calcium conce tration, as would be expected, simultaneously with a reversal of the myocardi depression despite persistent hyponatremia. Protection by calcium again the arrhythmias seen in CO_2 washout has similarly been reported by Goo and Miller.[15]

The arterial oxygen saturation is reported in TABLE 5. Of particular i portance is the improvement in the saturation that occurs with the correcti of the hypercapnic acidosis. This change, present in specimens taken 1 mi after the infusion of THAM was begun, rules out the possibility that the my cardial deterioration was secondary to anoxemia. The nature of the desatu tion seen in some dogs after 4 hours of hypercapnic acidosis is not immediate vious. The data of Holmdahl[16] suggest that the Bohr effect should not ount for desaturation with inspired oxygen concentrations as high as 60 r cent, and the absence of desaturation in some of the studies tends to conm this. If, as seems likely, the arterial hypoxemia is due to disturbance of ntilation-perfusion relationships in the lung (shunting) it is remarkable that

TABLE 3
EFFECT OF THAM ON PLASMA SOLUTES

og No.	Control				CO_2 4 hour				THAM				Calcium			
	Na^+ mEq/l.	K^+ mEq/l.	Cl^- mEq/l.	Glucose mg. %	Na^+ mEq/l.	K^+ mEq/l.	Cl^- mEq/l.	Glucose mg. %	Na^+ mEq/l.	K^+ mEq/l.	Cl^- mEq/l.	Glucose mg. %	Na^+ mEq/l.	K^+ mEq/l.	Cl^- mEq/l.	Glucose mg. %
1	—	2.9	—	—	147	7.6	89	—	100	9.0	85	—				
2	148	3.3	101	—	153	7.4	—	—	112	9.5	88	—				
3	153	4.1	114	128	154	11.2	108	301	122	11.6	100	181				
4	148	3.4	110	—	155	8.3	—	—	121	8.0	96	—				
5	159	3.0	110	136	148	8.7	—	490	108	9.0	85	364				
6	143	3.9	108	98	141	8.2	99	372	103	8.1	84	198				
7	148	4.1	115	109	146	7.8	104	357	108	9.3	93	186	—	9.4	—	—
8	151	3.9	110	183	152	8.3	103	368	110	10.5	96	291	115	11.1	99	278
9	151	3.6	109	66	153	8.7	100	316	101	11.0	85	225	97	11.0	89	252
10	145	4.3	113	136	144	9.9	101	270	117	11.9	101	260	105	9.2	93	236
Mean	149	3.7	110	122	149	8.6	101	298	109	9.8	91	244				

TABLE 4
FECT OF THAM ON TOTAL PLASMA CALCIUM CONCENTRATION* DURING HYPERCAPNIA

Dog No.	Control	CO_2 4 hour	THAM	Calcium
8	5.7	6.2	3.3	7.0
9	5.4	5.8	2.9	4.5
10	4.9	5.4	4.1	3.2
Mean	5.3	5.8	3.4	4.9

* mEq/l.

TABLE 5
EFFECT OF THAM ON ARTERIAL OXYGEN SATURATION* DURING HYPERCAPNIA

Dog No.	Control	CO_2 4 hour	1 min. THAM	5 min. THAM	Calcium
3	95.7	78.6	84.5	75.4	
4	97.7	72.8	99.4	98.4	
5	99.3	92.5	99.4	85.0	
6	99.7	99.4	98.9	99.8	
8	99.2	92.1		98.7	100
9	97.1	77.0		93.8	96.2
10	98.3	97.5		99.4	99.7
Mean	98.1	87.1		92.9	

* Per cent saturation.

this disturbance can be so rapidly corrected when arterial *p*H is restored towar normal.

Comment

Probably the principal conclusion that can be drawn firmly from this stu is the simple observation that correction of profound hypercapnic acidosis infusion of the amine buffer 2-amino-2-hydroxymethyl-1,3-propanediol d not result in evidence of ventricular hyperirritability. Asystole occurs wi out any evidence of a phase resembling that seen in the reversal of the hyp capnic state of CO_2 washout. This does not call into question, however, t conclusions as to the mechanisms underlying ventricular fibrillation report in previous studies. There are two principal divergences which make t comparison difficult.

First we must consider the effect of infusion of hypertonic solutions per on the circulation. Reported studies describe transient systemic hypotens following intravenous or intra-arterial injection of concentrated salt solution This has been, in general, attributed to systemic vasodilatation. The effe of nonelectrolyte hyperosmotic solutions is less consistent. Eliakin *et a* investigated cardiovascular responses to intravenous infusion of 20 per ce sodium chloride (3.4 *M*) in single doses of 1 ml./kg. These authors report the occurrence of bradycardia and apnea, with a marked increase in the p monary venous to left atrial pressure gradient. Control infusions of 50 cent glucose (2.8 *M*) were inactive, however, and the effect was theref attributed by them to a direct action of the sodium ion. Bellet *et al.*[19] stud 1 molar sodium lactate which, in doses of 0.6 to 0.8 ml./kg./min., resulted ECG changes (depressed T waves, nodal rhythm), reduction of heart fo (strain gauge arch) and fall in blood pressure. The changes seen were li wise believed unrelated to hypertonicity as control infusions of 2 molar sorbi were without effect. Read *et al.*[20] on the other hand describe vasodepres phenomena with 50 per cent glucose, although not with hypertonic urea. any event, direct cardiac depression and arrhythmias do not appear attrib able to hypertonicity alone.

Second, there is a sharp fall in plasma sodium concentration in the pres study that is not seen during CO_2 washout. Although it is not possible assess how much of the hyponatremia is dilutional and how much due to int cellular leakage of sodium, the change is in any case sufficiently profound impair seriously the function of cardiac muscle. Replacement of sodium is motically by THAM in Ringer's solution is known[21] to resemble the effe of calcium and to increase the twitch tension of isolated cardiac muscle stri The results of these authors suggest, however, that relaxation may be mechanical function most affected by replacement of sodium by THAM, solutions containing the latter produced contracture with constant concent tions of calcium and slight potassium excess. The interpretation of this re tionship, with respect to our data, is made still more puzzling by the respo we found to calcium. Since calcium and sodium are considered to be ant onists and the sodium antagonism is not displayed by THAM, further incre in calcium concentration would seemingly not be expected to have so salut an effect.

Directly in point here is also the recent report of Young *et al.*,[22] who in the
g heart-lung preparation employed hypertonic (1 molar) sodium bicarbonate
increase the *p*H during short-term hypercapnic acidosis accompanied by
tassium chloride infusion. In this preparation, in which ECG deterioration
uld be expected to occur with CO_2 washout, the correction of the acidosis
s unaccompanied by evidence either of heightened irritability of the heart
of myocardial failure. The plasma sodium concentrations following the
fusion of bicarbonate ranged from 172 to 220 mEq/l. In a sense, it seemed
if high sodium concentrations during acute correction of the hypercapnic
idosis without correction of the hypercapnia protected the heart from both
rhythmia and myocardial failure. This result is in direct contrast to the
uation in our preparation, in which hyponatremia of severe degree prevailed.
e are currently planning investigation of the response to molar sodium car-
nate infusion following 4 hours of hypercapnia in an attempt further to
rify this point. In addition, the correction of the myocardial failure by
lcium during persistence of hyponatremia remains to be explained.

Conclusions

The phenomenon of posthypercapnic ventricular fibrillation was not repro-
ced when hypercapnic acidosis was corrected by the rapid infusion of 2-amino-
hydroxymethyl-1,3-propanediol in dogs. The myocardium rather became
podynamic and asystole supervened. The administration of calcium chlo-
le at the termination of the infusion could reverse these changes.

Plasma solute changes accompanying the infusion were discussed, and
nilarities to and differences from the posthypercapnic phenomenon shown
exist. Despite the collection of much information, the mechanism underly-
g posthypercapnic ventricular arrhythmias remains unclear.

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EFFECTS OF 2-AMINO-2-HYDROXYMETHYL-1,3-PROPANEDIO ON CO_2 ELIMINATION AND PRODUCTION IN NORMAL MAN

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After a long and distinguished career in Warburg apparatus and in fish tra port vats, Tris buffer, 2-amino-2-hydroxymethyl-1,3-propanediol, was p posed for treatment of respiratory acidosis.[1] Nahas employed this compou also called THAM, to counteract the effects of carbon dioxide accumulat in dogs placed on diffusion oxygenation. Brinkman and his associates[2] ha studied the effects of this compound in patients with respiratory acidosis fr emphysema and carcinoma of the lung. A number of chronic and acute to ity studies in animals indicated no lasting ill effects could be expected from administration.

We were particularly interested in the possibility of eliminating carbon di ide via the kidneys in combination with Tris buffer. Moreover, we w intrigued by the report that the ventilation was reduced during its administ tion and the statement that during THAM administration, changes in min ventilation are related to changes in arterial blood pH.[3]

Since some measure of hypoxic drive could be expected with hypoventilat on air, we designed a study that would test the above hypotheses in the sence of hypoxia. The aim of the study was to produce hyperventilation administering carbon dioxide in the air inspired and then to compensate exac for the inspired carbon dioxide by infusion of Tris buffer, returning the vent tion to the original level of ventilation on room air. Since the carbon diox concentration in the inspired air was not to exceed 4 per cent, the inspi mixture would not contain less than 20 per cent oxygen and no hypoxia co ensue.

Method

This series of experiments was performed on young volunteer subjects, a 24 to 32, who were healthy according to history and physical examinati The subjects were trained to the extent that they previously had participa in respiratory physiology studies. The subjects inspired via a mouthpi through a low resistance valve from either a spirometer containing compres air or, on turning a valve, from another containing the carbon dioxide-air m ture. Exhalation was through another low resistance valve into a self-emp ing servospirometer triggered by inspiration to dump its contents into one two Tissot spirometers. The carbon dioxide-air mixtures inspired had p viously been analyzed by Scholander analysis. Inspired and expired gas w continuously monitored with an infrared carbon dioxide analyzer using ti mpling. Expired air collected over 5-min. intervals in the Tissot spirometers is sampled for Scholander analysis, but rapid analysis by an infrared carbon oxide analyzer and a paramagnetic oxygen analyzer were so accurate that in ost studies only occasional checks by Scholander analysis were done.

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An indwelling urethral catheter was employed to obtain urine for analysis d output measurement. A Riley-Cournand needle in the femoral artery ovided specimens of arterial blood. An 18-gauge needle in a forearm vein as used to administer either 5 per cent glucose solution or a 0.3 molar solution Tris buffer in 0.03 molar saline and 0.005 molar potassium chloride. The te of administration was controlled by suspending the bottle from a balance d weighing frequently.

The electrocardiogram was continuously monitored using Standard Lead II. ood pressure was taken intermittently during the course of the study. The bject was observed constantly for overt symptoms and was occasionally ked questions regarding his condition answerable by signs.

On arterial blood samples, the *p*H, carbon dioxide content, carbon dioxide nsion, serum glucose, sodium, potassium, calcium, chloride, and inorganic

TABLE 1

DOSE SCHEDULE OF TRIS BUFFER

Subject	Weight (kg.)	Infusion rate (m*M*/min.)	Total dose	
			Total m*M*	m*M*/kg.
A	88.5	5.13	252	2.85
B	83.2	5.28	339	4.1
C	71.6	10.53	632	8.8
D	75.5	7.53	256	3.0

osphate were determined. In urine samples, the *p*H, carbon dioxide content, is buffer, urea, glucose, sodium, potassium, chloride, and phosphate were termined. The infusion rates and quantities of Tris buffer administered e given in TABLE 1. The first two subjects, A and B, were given 2.3 per cent rbon dioxide in air, and the last two subjects, C and D, 3.4 per cent carbon oxide in air.

In the usual course of a study, the subject ingested 0.5 to 1 l. of water per ur the evening prior to the study and, in the morning, although fasting, continued ingesting a water load at the same rate up to the hour of the study. hen all was ready, the subject, reclining on a cot with his nose clamped shut, sumed the mouthpiece and the control period on air was started. During s period the 5 per cent glucose solution dripped very slowly. Several blood mples were collected and, at the end of the period, the urethral catheter was mped and the bladder was emptied. Similar sampling was carried out while eathing the carbon dioxide-air mixture in the next period, which continued a minimum of 30 min. and was extended to nearly 1 hour in the second subt who did not show stability in minute ventilation at first on this mixture. nile the subject continued to inspire the carbon dioxide-air mixture, the inion of Tris buffer solution was begun. Blood sampling was done more frequently, and urine was collected in the middle and at the end of this per The first three subjects had the infusion of Tris buffer and the carbon dio inhalation stopped simultaneously, and a period of recovery lasting an hou more was begun. The fourth subject was continued for half an hour on carbon dioxide mixture before the recovery period on air was begun.

Results

The situation existing in the pertinent respiratory parameters and uri excretion at the end of each period in the several subjects provides a rough

TABLE 2

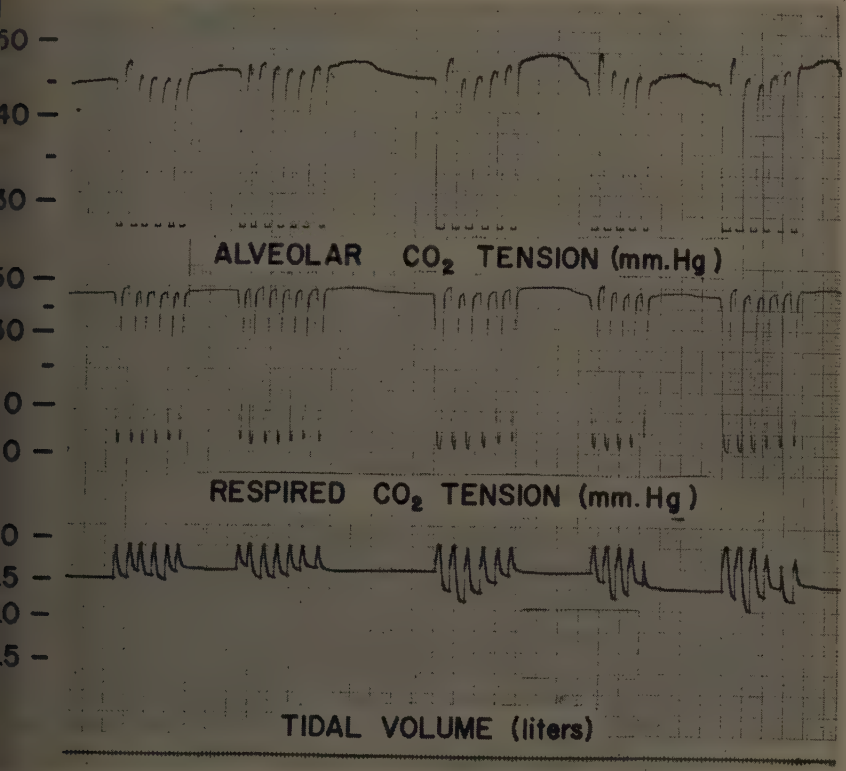
Values of Respiratory Parameters at the End of Control, Carbon Dioxi Breathing, THAM Infusion, and Recovery Periods. Urinary Excretion o THAM and Carbon Dioxide for Last Half of Infusion Period or All of Other Periods

Subject	Period	Duration (min.)	V_T (l.)	f/min.	V_E (l./min.)	V_A (l./min.)	$P_{A}CO_2$ (mm. Hg)	V_{CO_2} (cc./min.)	*p*H	CO_2WB (m*M*/l.)	$P_{a}CO_2$ (mm. Hg)	O. Sat. (%)	Urine CO_2 (mM)
A	Control	29	1.773	5	8.93	5.9	38	253	7.42	20.6	37	99	3.4
	CO_2 (2.3%)	35	1.538	7	11.17	7.6	43	238	7.40	21.1	39	97	2.1
	CO_2 + THAM	50	0.900	11	9.81	6.3	43	198	7.48	24.8	39		10.1
	Recovery	60	0.803	11	9.02	5.8	40	264	7.52	23.1	34	99	40.0
B	Control	35	0.835	7	5.76	3.7	44	187	7.33	22.7	50	93	2.9
	CO_2 (2.3%)	54	0.900	11	9.21	6.4	45.5	196	7.35	22.5	48	92	2.8
	CO_2 + THAM	63	0.490	12	6.66	3.3	50	131	7.42	29.7	55	96	31.1
	Recovery	98	0.570	12	6.58	3.6	45	186	7.40	27.1	52		34.8
C	Control	40	0.850	9	8.52	5.8	30	203	7.41	21.4	40	100	4.5
	CO_2 (3.4%)	35	0.950	13	13.30	7.0	43	167	7.33	22.7	48	98	3.7
	CO_2 + THAM	60	0.375	17	6.39	1.6	41	37	7.53	33.9	47	83	43.4
	Recovery	155	0.410	17	7.26	2.9	51	174	7.37	30.0	57	93	24.0
D	Control	45	1.162	6	6.28	4.7	36	223	7.34	19.5	42	100	21
	CO_2 (3.4%)	30	1.434	8	12.63	9.5	42.5	230	7.31	20.4	46	100	0.4
	CO_2 + THAM	30	1.075	7	8.65	5.7	43	129	7.39	24.9	47	98	15.0
	CO_2	30	1.129	10	11.72	8.4	44.5	213	7.38	24.8	48	95	19.1
	Recovery	115	0.859	8	6.70	4.7	39	214	7.38	24.5	48	96	19.0

of the changes observed (TABLE 2). The change from air to the carbon diox air mixture produced a rise of 2 to 6 mm. Hg. in the alveolar and arterial *p*C With this rise the tidal volume increased and then minute ventilation ro to 6 l./min. The *p*H decreased 0.02 to 0.08 U., and the carbon dio content rose at most 1.3 mmole/l.

On starting the buffer solution a transient decrease of 3 mm. pCO_2 was served in the expired alveolar air, and a few breaths later a decrease in t volume occurred. This decrease in tidal volume reduced the minute vol to nearly the control level in the first 2 subjects infused at a rate of 5 mmo min., which should correspond to 100 cc. of carbon dioxide/min. Their car dioxide load was 150 cc. of carbon dioxide at the control level of minute ven tion.

In the third subject, 3.4 per cent carbon dioxide in air was to be employed d the carbon dioxide load was expected to be 200 cc./min. over and ove intrinsic production. To balance this carbon dioxide load an infusion e of 10 mmoles/min. was computed. Instead the infusion should have been nsidered to titrate the intrinsic carbon dioxide production of 175 cc./min. eoretically the buffer should more than counter this production rate and ould leave the subject without an excuse to breathe except in response to



IGURE 1. Periodic breathing of subject C at the height of THAM effect. Oxygen satura-, 83 per cent; P_aCO_2, 47 mm. Hg; *p*H, 7.53.

xia. The subject did in fact maintain his ventilation up to the last 10 min. he period, when he began to exhibit periodic breathing (FIGURE 1), and the erial oxygen saturation decreased to 83 per cent.

s in the previous subjects this subject returned to about the control minute ume and carbon dioxide output rate when the infusion was stopped and air ne inspired. The tidal volume did not increase to the control level in any ject and their respiratory rates stayed elevated. Periodic breathing pered in this subject and continued, accompanied by retching, weakness, and nolence for 24 hours.

Following this episode both the rate of infusion and total buffer were creased in the fourth subject to 7.5 mmoles/min. and 225 mmoles total In this subject, who also was given 3.4 per cent carbon dioxide in air, the eral results were similar to the previous studies (FIGURE 2). However

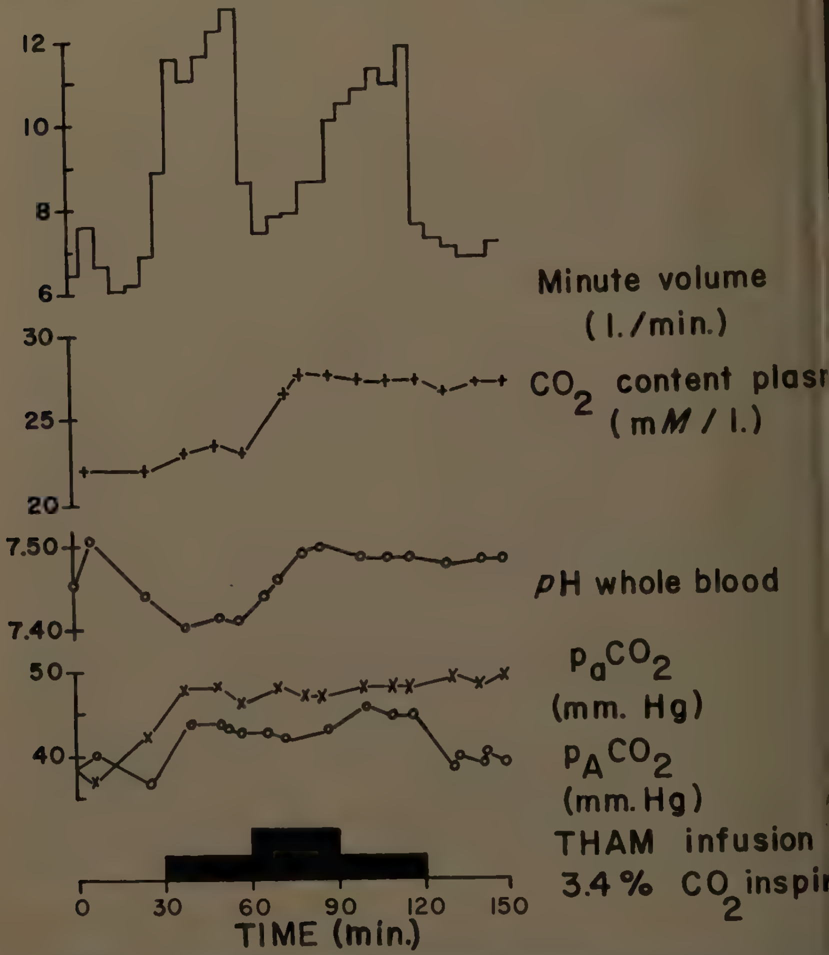
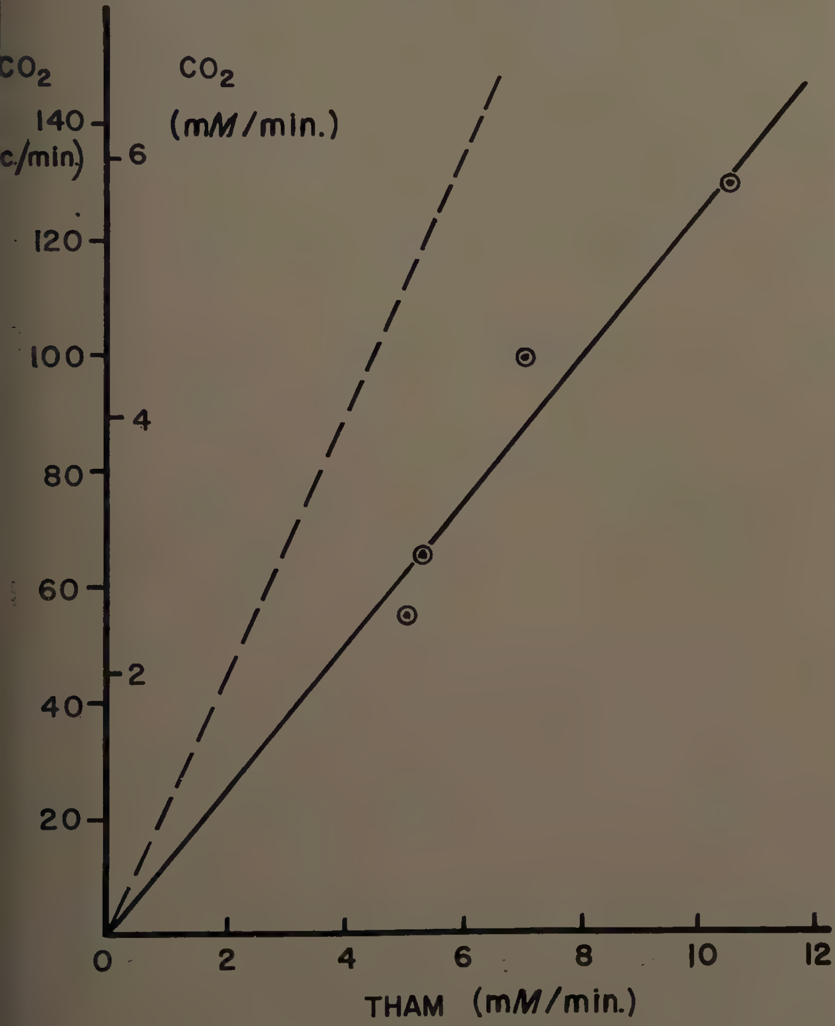


FIGURE 2. Changes in respiratory parameters during study on subject D. Parall in carbon dioxide content and *p*H with essentially constant pCO_2 in arterial blood and al air during THAM infusion.

previously mentioned, carbon dioxide breathing was continued after th fusion of buffer was stopped. On discontinuing the buffer a transient r the alveolar pCO_2 was observed about the same in magnitude as the dec seen just after it was started. An increase in the minute volume occurre net carbon dioxide output rate returned to its previous value. At this the *p*H was 0.07 units higher than in the carbon dioxide period befor sion. On removing the carbon dioxide load, the minute volume returned he control level.

alculation of the carbon dioxide output rate for each Tissot collection al- ed us to follow the retention rate during the infusion period. The rate of



IGURE 3. Relation of rates of infusion of THAM and rate of carbon dioxide retention. ned line represents equality in rates.

carbon dioxide accumulation was proportional to the rate of infusion (FIG- 3). However, the accumulation was not on an equimolar basis, but indi- d only half a mole of carbon dioxide was retained per mole of buffer in- d.

he rate of urinary excretion of the buffer was dependent on the urinary output since the concentration was about 80 mmoles/l. during flows of 5000 per hour or over. The concentration of buffer in the urine reached alm 200 mmoles/l. when urine output fell to 200 cc. per hour or less. Recov was 62, 62, 78, and 75 per cent in the 4 subjects over a 72-hour period.

During the infusion, carbon dioxide was excreted in urine in equimolar p portion to the amount of buffer excreted. During the recovery period w urine output was high, carbon dioxide was also excreted in an almost equim ratio. As soon as the urine output decreased and concentration of buffer r gan, carbon dioxide was excreted in increased concentration but not in e molar proportions. The excretion of carbon dioxide was not followed m than two hours beyond the infusion period.

There were no remarkable changes in electrolytes other than bicarbona No changes in urea blood level or urinary excretion were observed. The m interesting and, in retrospect, most alarming change was the decrease in ser

TABLE 3

DEPRESSION OF SERUM GLUCOSE AND PHOSPHATE LEVELS DURING TRIS BUFFER INFUS

Subject	Control		CO_2	CO_2 THAM Period 1	CO_2 THAM Period 2	CO_2	Recov
A	Glucose (mg. %) Phosphate (mg. %)	91 2.9	97 3.3	78 3.1	74 2.9		75 3.
B	Glucose (mg. %) Phosphate (mg. %)	102 3.5	111 3.5	90 3.2	80 2.9		86 3.
C	Glucose (mg. %) Phosphate (mg. %)	101 3.3	89 3.6	55 2.6	52 2.3		79 1.
D	Glucose (mg. %) Phosphate (mg. %)	87 3.5	80 3.7	77 3.6	76 3.2	65 3.3	80* 3.

* Fifty cc. of 50 per cent glucose at end of CO_2 period.

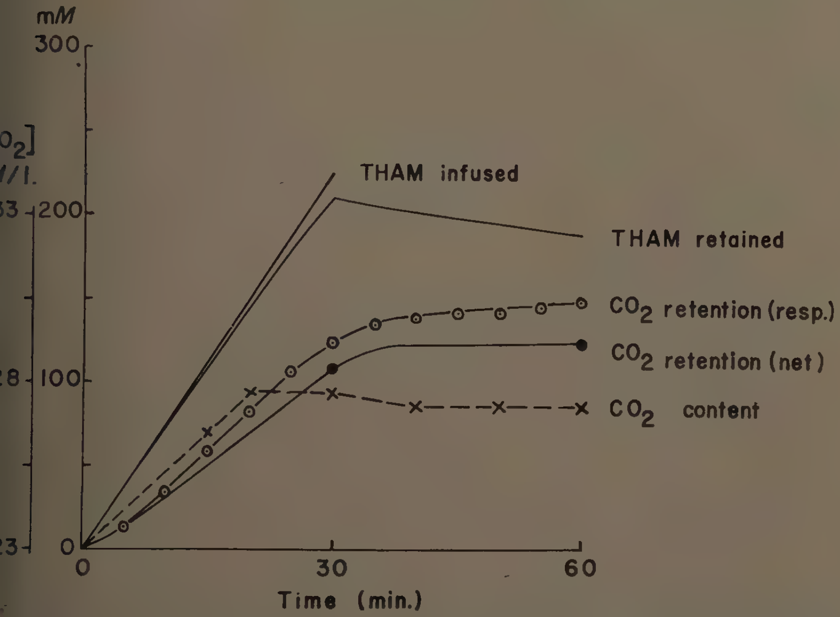
glucose level (TABLE 3). The greatest decline was in the third subject wh glucose level dropped from 101 to 52 mg. per cent during the infusion of buf The phosphate level also decreased from 3.4 to 2.3 mg. per cent during period of hypoglycemia.

Both *p*H and carbon dioxide content rose during the infusion. The in ment was between 0.4 and 1.0 mmole of carbon dioxide content for each hundredth of a *p*H unit. For the first quarter hour, carbon dioxide cont increased with the accumulating dose of buffer and carbon dioxide retai (FIGURE 4). However, during the remainder of the infusion, carbon dio content increased less rapidly and so did *p*H. The carbon dioxide space co be computed and appeared to increase up to 25 l. as the retained carbon dio distributed itself through the body's compartments.

Unhappily, methods for determination of buffer concentration in bl proved unreliable. We are therefore unable to report on the distributio this material in the body. For the same reason the data on kidney func we hoped to obtain are not available.

Discussion

hese experiments seem to indicate that respiratory acidosis could be treated
the administration of Tris buffer. In a patient already breathing only
n hypoxic drive, further carbon dioxide accumulation should not occur
ing the administration of buffer. If urine flow could be kept up, carbon
xide in almost equimolar proportions could be removed. To reduce the
bon dioxide by 10 mmoles/l. would require about 250 mmole of Tris buffer
an equimolar basis. About 60 per cent of this would be excreted in 24 hours
l most of the remainder in the next 2 days. To avoid accumulation and the



IGURE 4. Relation of changes in carbon dioxide content and the accumulated carbon
ide retained and THAM infused. Carbon dioxide space was 15 l. at 15 min., 22.5 l. at
in., and 25 l. at 60 min.

oglycemic syndrome, the frequency of use should probably be restricted.
a means of counteracting the depressant properties of carbon dioxide in
inating respiratory acidosis from emphysema, the buffer might be of use
ip the balance when other measures were also being employed. The re-
tions on the total dosage prevent its use as the sole agent in this syndrome.
t present no routine use of the buffer solution to titrate the carbon dioxide
luction of man is contemplated. Since two moles of buffer are needed per
e of carbon dioxide, the complete neutralization of the carbon dioxide pro-
ion would require an excessive dosage over a period as short as half an
r. To maintain the *p*H in a paralyzed apneic individual would not require
reat a dosage and might be feasible for short periods. Here both $p\mathrm{CO}_2$
carbon dioxide content would rise during the accumulation of carbon diox-

In either case, the need for maintenance of the *p*H or pCO_2 at the nor
level is not documented. For periods of up to 10 min., the usual maxim
time of endoscopy, an accumulation of no more than 2 l. of carbon dioxide
be expected. This equals about 100 mmoles of carbon dioxide and, in a carb
dioxide space of 10 l., would cause a rise of 10 mmoles/l. in carbon dioxide c
tent and 0.10 to 0.25 units drop in *p*H. Jooste[4] maintained more than
patients in apneic oxygenation for up to 10 min. without mishap. Che
and Chambers reported 50 similar cases of apneic oxygenation.[5] One of
has employed the technique routinely for endoscopy after hyperventilat
with oxygen without untoward results. Frumin *et al.*,[6] in search of to
effects, have prolonged apnea in man as long as three quarters of an hour
have observed P_aCO_2 as high as 160 mm. Hg. without cardiac irregularit
On the other hand, Altschule and Sulzbach[7] encountered marked EKG chan
in apneas lasting 10 to 20 min., and Brown and Miller[8] reported ventricu
fibrillation in the posthyperapneic period. Finally, Graham *et al.*,[9] us
cyclopropane to control the convulsions seen at 30 to 40 per cent carbon di
ide, carried dogs to carbon dioxide levels of 60 to 70 per cent where spontane
respirations returned. The animals were maintained for 1 hour at this conc
tration and returned to a normal tension of carbon dioxide while breath
oxygen without incident.

The data obtained in this study indicates that neither *p*H or P_aCO_2 are dir
regulators of ventilation throughout their full range of values. In particul
*p*H can be markedly increased without altering the level of ventilation. In
first two subjects *p*H was increased by 0.07 units or more while P_aCO_2
comparable in the control and recovery periods. The minute ventilati
however, was increased slightly, while alveolar ventilation was unchanged. T
fourth subject in the control and recovery periods while breathing air had
same alveolar ventilation while the *p*H and P_aCO_2 were both higher in
recovery period. During the carbon dioxide breathing periods, he had a
per cent lower ventilation in the period after the buffer infusion when the
was 0.07 units higher and the P_aCO_2 2 mm. higher.

These *p*H differences with no important ventilation differences imply t
the subjects were below the apneic threshold for *p*H. This apneic thresh
for *p*H has been demonstrated by Bjurstedt[10] and by Gernandt.[11] This apn
threshold for *p*H probably is based on the same underlying mechanism as
apneic threshold for carbon dioxide[12] and the two should be interrelated. V
tilation was maintained in all but the third subject probably by the consci
drives suggested by Fink.[13] The third subject was obviously toxic from
buffer and was operating on hypoxic drive.

These comparisons of the ventilation during "steady state" conditions m
be contrasted with ventilation during buffer infusion. The buffer solution w
a *p*H of 10.5 may be considered to titrate and neutralize carbonic acid produ
by the metabolic processes. The first result of infusion noticed was reduct
in the P_aCO_2. Then a reduction in ventilation occurred that reduced
output of carbon dioxide eliminated through the lungs. These results are
the opposite direction to the changes seen on infusion of HCl solution. So
tions of HCl as well as solutions of bicarbonate, rebreathing or direct intra

s carbon dioxide gas infusion elevate the P_aCO_2 and produce hyperventila- n in greater or less degree during their application. This hyperventilation he struggle of the body to eliminate metabolic carbon dioxide as well as t carbon dioxide being liberated from plasma bicarbonate by acid. Hypo- tilation during Tris buffer infusion reflects the easing of the need for elimina- n of all metabolic carbon dioxide by the lungs.

The amount of carbon dioxide retained during the infusion was less than ected. Only 55 per cent of the carbon dioxide theoretically expected to ct with the buffer accumulated during the infusion. The ionization of fer should theoretically be about 80 per cent complete at a *p*H of 7.4.[14] is type of discrepancy also is observed in the infusion of HCl.[15,16] The planation suggested was that intracellular buffers are called into play. In data the rapid increase in the carbon dioxide dilution space of the accumula- g metabolic carbon dioxide suggests that plasma and interstitial fluid equili- te rapidly and some accumulation in the cells had begun in less than an hour. bably buffer had moved into the interstitial fluid and the cells, since ac- mulation in the cell without entry of buffer requires increased back pressure m carbon dioxide in the interstitial fluid.

Except for the third subject, who was probably toxic, the subjects returned normal pCO_2 levels in the recovery period but left their *p*H high. These jects at the end of the infusion continued to excrete alkaline urine and re- ned to the preinfusion *p*H status slowly. From the decrease in bicarbonate he urine during the first portion of the recovery period the amount of carbon xide eliminated through the kidneys is estimated to equal that accumulated ing the infusion. However, in the absence of buffer the accumulation uld also be eliminated through the kidneys. As shown by Pitts and Lots- ch[17] and, later, by Thompson and Barrett,[18] excess bicarbonate is eliminated ough failure to reabsorb the bicarbonate ion present in the glomerular filtrate excess of a threshold concentration. In our study the effect of increasing bicarbonate is not separated from the effect of the presence of the buffer. ese data do not indicate whether, in a patient who had accommodated to an vated bicarbonate level, the kidneys would eliminate bicarbonate without se in bicarbonate level. Both in the patients of Brinkman *et al.*[2] and in a gle patient to whom we have given the buffer, hypoventilation and accumula- n of CO_2 occurred despite a high initial P_aCO_2 and low P_aO_2. The ques- n remains whether the buffer serves any useful function in the urinary ex- tion of bicarbonate except saving of cations.

These effects of carbon dioxide breathing and Tris buffer administration d to a concept of ventilatory changes to maintain carbon elimination from respiratory center that depends on the back pressure of carbon dioxide in interstitial fluid, in the capillary blood, and, finally, in the alveolar air. so far as changes in *p*H and bicarbonate concentration affect the back pres- e of pCO_2, these parameters also will influence the respiratory center. en the outflow rate of carbon dioxide from the center is too uninhibited ctivity of the center results and a carbon dioxide or *p*H apneic threshold is erved.

This concept, essentially that of Gesell,[19] permits wide ranges in *p*H and bicarbonate ion concentration at the same ventilation but small ranges in *pt* as observed in alveolar air and arterial blood. The regulation of *p*H is lef the cells themselves through changing their acid excretion with the assista over-all of the kidneys. The bicarbonate concentration is the resultant of foregoing regulations and excretion of the excess bicarbonate by the kidn This concept provides a satisfactory explanation of the observed results t short and long term of increased acid administration or retention, or, on other hand, of changes seen in the alkalosis acquired by alkali ingestion infusion.

Summary

Four volunteers were infused with Tris buffer while breathing 2.3 or 3.4 cent carbon dioxide in air. Part of the carbon dioxide produced accumula during the infusion, half a mole being retained for each mole of buffer. P_aCO_2 was maintained constant by a decrease in ventilation through reduct in the tidal volume while carbon dioxide content and *p*H were elevated. stopping the buffer infusion, ventilation increased again and the accumula of carbon dioxide stopped. During the infusion, carbon dioxide and bu were eliminated in equimolar proportions by the kidneys at a concentra of 80 mmoles/l. With lower urine output rates, the buffer was excrete higher concentrations but less carbonate was excreted. Sixty per cent of buffer was recovered in 24 hours, and as much as 78 per cent in 72 hours.

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ACID-BASE CHANGES IN THE CEREBROSPINAL FLUID FOLLOWING RAPID CHANGES IN THE BICARBONATE/CARBONIC ACID RATIO IN THE BLOOD*

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of Physicians and Surgeons, New York, N.Y.

Equilibration of dissolved CO_2 between blood and cerebrospinal fluid (C is a rapid process. However as shown by Robin *et al.*,[1] H^+ and HCO_3^- equilibrate slowly between the two compartments. This paper deals wit acid-base relationship between blood and CSF in dogs, following rapidl duced changes in the arterial bicarbonate/carbonic acid ratio. It was felt such a study could throw some light on the acid-base relationships of diffe body fluid compartments when different bases are added to the blood wit without a concomitant acid load.

Method

Eighteen medium-sized mongrel dogs (11 to 22 kg.) were used. Anest was induced by Pentothal (40 to 50 mg./kg. body weight) injected intr nously. After endotracheal intubation with a cuffed tube, the animals mechanically ventilated with pure oxygen. Succinylcholine was used complete neuromuscular block throughout the experiments.

In one series of experiments, performed throughout with constant vol ventilation, intravenous infusions of either 0.3 *M* tris(hydroxymethyl)am methane (Tris or THAM), or 0.3 *M* $NaHCO_3$, or 0.2 *M* Na_2CO_3 were given rate of 0.5 ml./kg. body weight per min. for one-half hour. There were 3 of each group in this series. In another series, solutions of 0.4 *M* THA of 0.2 *M* Na_2CO_3 were administered at a rate of 1.0 ml./kg. body weight min. to animals submitted to a concomitant hypercapnic load during min. of apneic oxygenation. Three dogs were used in each of the two gr in the second series and, in addition, 3 dogs were submitted to one-half ho apneic oxygenation without infusion of a buffer.

Polyethylene catheters were inserted into the femoral artery and vein, arterial catheter being connected to a Statham pressure transducer. An gauge needle was inserted in the cisterna magna and connected by a polyet ene catheter to a Statham transducer. The pressures were recorded on a born polyviso recorder.

The half-hour test period, when different buffer solutions were infused, always preceded by at least one-half hour of mechanical ventilation.

Samples (0.5 ml.) of arterial blood and CSF were taken immediately b and at the end of the period of infusion. A third set of samples was t

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alf hour after the period of infusion in the first series, and one hour after- in the second series.

terial blood and CSF pHs were measured anaerobically at body tempera- CO_2 content in plasma and CSF was measured by the Natelson[2] micro- meter method as modified by Holaday and Verosky.[3] $PaCO_2$ and HCO_3^- calculated by the Henderson-Hasselbalch equation, using a pK' of 6.10[4] lasma, a pK' of 6.13[5] for spinal fluid, and a CO_2 solubility coefficient of 01[6] and 0.0312[7] respectively.

TABLE 1

SION OF SODIUM BICARBONATE* IN THREE DOGS VENTILATED AT CONSTANT VOLUME

Wt. (kg.)	Time	Arterial blood					CSF				
		CO_2 (mM/l.)	pH	pCO_2 (mm. Hg)	HCO_3^- (mM/l.)	Mean pressure (mm. Hg)	CO_2 (mM/l.)	pH	pCO_2 (mm. Hg)	HCO_3^- (mM/.l)	Mean pressure (mm. H_2O)
22	0′	18.66	7.36	32.8	17.67	180	19.73	7.36	34.8	18.65	100
	30′	35.26	7.50	44.6	33.92	183	22.22	7.34	41.3	20.93	160
	60′	28.69	7.43	42.1	27.42	180	23.56	7.39	39.1	22.34	72
11	0′	19.16	7.33	35.0	18.01	112	19.70	7.31	39.1	18.48	138
	30′	33.58	7.47	45.6	32.21	110	23.11	7.29	47.8	21.62	108
	60′	26.65	7.41	40.7	25.43	102	22.15	7.36	39.3	20.92	60
18	0′	17.37	7.44	24.9	16.62	129	18.17	7.39	30.0	17.20	56
	30′	33.56	7.62	32.2	32.59	154	19.73	7.31	38.7	18.52	62
	60′	26.10	7.54	31.0	25.77	148	20.40	7.38	34.6	19.32	50
	0′	18.40	7.38	30.9	17.43	140	19.20	7.35	34.6	18.11	98
	30′	34.13	7.53	40.8	32.91	149	21.69	7.31	42.6	20.36	110
	60′	27.15	7.46	37.9	26.21	143	22.04	7.37	37.7	20.86	61

.5 ml./kg./min. of 0.3 M $NaHCO_3$.

ey: 0′ = control (M.V.); 30′ = after 30′ $NaHCO_3$ infusion; 60′ = 30 min. after end of ion.

Results

e results of the first series of experiments are shown in TABLES 1 to 3. The ion of 0.3 M $NaHCO_3$ (TABLE 1) during constant volume mechanical ilation resulted in an alkaline shift in the blood of 0.15 pH units, due to a ortionally smaller increase in $PaCO_2$ than in HCO_3^-. The average rise aCO_2 was 10 mm. Hg., whereas the average increase in HCO_3^- was 15.5 ./l. On the other hand, in the CSF there was an acid shift of 0.04 pH , due to a proportionally smaller increase in HCO_3^- than in $PaCO_2$. There ise in $PaCO_2$ was of the same order as in the blood, but the rise in HCO_3^- nly one seventh of that in the blood. During the one-half hour following ion, the HCO_3^- continued to rise slowly in the CSF, although it immedi- started to decrease toward normal values in the blood.

hen Na_2CO_3 was infused (TABLE 2) during constant volume mechanical lation, there was essentially no change in $PaCO_2$ and $P_{CSF}CO_2$. There an average increase of 13.3 mEq./l. of HCO_3^- in the blood, but only an increase of 1.0 mEq./l. of HCO_3^- in the CSF. The marked alkaline shif 0.21 *p*H units in the blood was therefore not seen in the CSF where the was essentially unchanged, with a slight increase in two experiments an slight decrease in one. Here also the HCO_3^- continued to rise in the during the period following infusion.

With infusion of THAM (TABLE 3), an alkaline shift occurred in the CSF well as in the blood, in all three experiments, with an average of 0.06 and *p*H units respectively. Also there was a consistent slight decrease in P_{CC} the CSF as well as in the blood. The HCO_3^- increased with an average of mEq./l. in blood, whereas it remained essentially unchanged in the CSF.

TABLE 2

INFUSION OF SODIUM CARBONATE* IN THREE DOGS VENTILATED AT CONSTANT VOL

Dog (No.)	Wt. (kg.)	Time	Arterial blood					CSF				
			CO_2 (m*M*/l.)	*p*H	*p*CO_2 (mm. Hg)	HCO_3^- (m*M*/l.)	Mean pressure (mm. Hg)	CO_2 (m*M*/l.)	*p*H	*p*CO_2 (mm. Hg)	HCO_3^- (m*M*/l.)	M pre (n H
96	20	0′	21.08	7.50	26.6	20.28	107	20.19	7.46	29.0	19.29	1
		30′	29.14	7.61	28.6	28.28	129	19.69	7.45	28.5	18.81	1
		60′	25.30	7.49	32.9	24.31	118	22.55	7.38	38.0	21.37	
919	20	0′	19.84	7.39	31.9	18.88	145	21.05	7.33	40.0	19.80	1
		30′	30.59	7.58	32.1	29.62	127	21.76	7.35	39.4	20.53	1
		60′	25.37	7.51	31.4	24.43	112	22.47	7.38	37.9	21.29	
920	10	0′	19.49	7.38	31.9	18.53	168	21.05	7.31	41.4	19.76	1
		30′	40.80	7.70	32.8	39.81	115	23.75	7.37	40.8	22.48	1
		60′	31.78	7.52	38.4	30.62	121	25.16	7.40	40.6	23.89	
Av.		0′	20.14	7.42	30.1	19.23	140	20.76	7.37	36.8	19.62	1
		30′	33.51	7.63	31.2	32.57	124	21.73	7.39	36.2	20.61	1
		60′	27.48	7.51	34.2	26.45	117	23.39	7.39	38.8	22.18	

* 0.5 ml./kg./min. of 0.2 *M* Na_2CO_3.

Key: 0′ = control (M.V.); 30′ = after 30′ Na_2CO_3 infusion; 60′ = 30 min. after en infusion.

There were no consistent or significant changes in arterial blood pressur CSF pressure in any of the three groups of the first series, except for a consis drop in CSF pressure after the infusion period in all three groups (TABLES 3).

The results of the second series, in which buffers were injected with a comitant acid load in the form of apneic oxygenation, are shown in TAB 4 to 6. During apneic oxygenation (TABLE 4) there was a total CO_2 reten with a mean increase of approximately 5 mm. Hg/min. in $PaCO_2$, and of 3.6 Hg/min. in $P_{CSF}CO_2$, when no buffer was injected. This acute respira acidosis caused a drop in blood and CSF *p*Hs to 6.8, as the somewhat higher in $PaCO_2$ than in $P_{CSF}CO_2$ was accompanied by a larger increase in blood HC than in CSF HCO_3^-.

When a 0.2 *M* solution of Na_2CO_3 was given (TABLE 5) at a rate of 1 ml./ min. during apneic oxygenation, the HCO_3^- increased by 34.6 mEq./l. in

TABLE 3

INFUSION OF THAM* IN THREE DOGS VENTILATED AT CONSTANT VOLUME

Wt. (kg.)	Time	Arterial blood					CSF				
		CO$_2$ (m*M*/l.)	*p*H	*p*CO$_2$ (mm. Hg)	HCO$_3^-$ (m*M*/l.)	Mean pressure (mm. Hg)	CO$_2$ (m*M*/l.)	*p*H	*p*CO$_2$ (mm. Hg)	HCO$_3^-$ (m*M*/l.)	Mean pressure (mm. H$_2$O)
12	0′	23.34	7.37	39.3	22.16	132	22.57	7.33	42.6	21.24	78
	30′	28.64	7.50	35.9	27.56	132	23.07	7.38	38.9	21.86	78
	60′	25.27	7.37	42.1	24.50	132	22.80	7.30	43.3	21.45	64
16	0′	19.53	7.36	33.8	18.52	162	21.49	7.42	31.1	20.52	80
	30′	26.11	7.62	25.1	25.36	143	22.63	7.48	28.9	21.73	90
	60′	22.31	7.42	33.2	21.31	144	21.74	7.32	31.2	20.52	60
22	0′	20.11	7.40	31.8	19.15	181	20.02	7.36	33.8	18.97	90
	30′	28.14	7.67	24.1	27.42	148	19.13	7.42	29.8	18.20	90
	60′	26.24	7.50	33.3	25.24	137	21.68	7.32	42.1	20.37	60
	0′	20.99	7.38	35.0	19.94	158	21.36	7.37	35.8	20.24	83
	30′	27.63	7.60	28.4	26.78	141	21.61	7.42	32.5	20.59	86
	60′	24.61	7.43	36.2	23.68	138	22.07	7.31	38.9	20.78	61

.5 ml./kg./min. of 0.3 *M* THAM.

y: 0′ = control (M.V.); 30′ = after 30′ THAM infusion; 60′ = 30 min. after end of on

TABLE 4

APNEIC OXYGENATION FOR ONE-HALF HOUR IN THREE DOGS

Wt. (kg.)	Time	Arterial blood					CSF				
		CO$_2$ (m*M*/l.)	*p*H	*p*CO$_2$ (mm. Hg)	HCO$_3^-$ (m*M*/l.)	Mean pressure (mm. Hg)	CO$_2$ (m*M*/l.)	*p*H	*p*CO$_2$ (mm. Hg)	HCO$_3^-$ (m*M*/l.)	Mean pressure (mm. HO$_2$)
22	0′	17.62	7.36	30.3	16.71	99	17.42	7.31	34.4	16.35	130
	30′	32.02	6.85	159.0	27.24	120	22.99	6.85	116.9	19.34	221
	90′	19.25	7.32	36.0	18.17	65	21.21	7.25	47.4	19.73	65
12	0′	13.40	7.38	21.8	12.74	143	16.06	7.39	26.5	15.23	97
	30′	30.18	6.75	180.7	24.74	178	26.38	6.74	165.4	21.22	260
	90′	14.88	7.40	23.3	14.18	110	22.10	7.40	35.9	20.98	32
12	0′	19.46	7.35	34.0	18.44	175	25.00	7.37	43.3	23.65	89
	30′	36.18	6.79	202.6	30.08	205	27.87	6.81	152.0	23.13	291
	90′	18.88	7.35	32.8	17.89	110	25.92	7.39	43.2	24.57	56
	0′	16.83	7.36	28.7	15.96	139	19.49	7.36	34.7	18.41	105
	30′	32.79	6.80	180.8	27.35	168	25.75	6.80	144.8	21.23	257
	90′	17.67	7.36	30.7	16.75	95	23.08	7.35	42.2	21.76	51

y: 0′ = control (M.V.); 30′ = end of apneic oxygenation; 90′ = 60′ after end of apnea .).

blood but only by 3.4 mEq./l. in the CSF. Since the carbon dioxide ten increased to almost the same degree in the blood as in the CSF, a marked ference between blood and CSF pHs arose. Due to almost proportional r in HCO_3^- and H_2CO_3 in the blood, the pH there was kept almost constant. the other hand, in the CSF the rise in P_{CO_2} was not accompanied by a sim rise in HCO_3^- and the pH dropped by an average of 0.28 pH U. When respiratory acidosis was buffered by an infusion of a 0.4 M solution of TH (TABLE 6) at a rate of 1 ml./kg./min., the average rise in HCO_3^- was 2 mEq./l. in the blood and 3.8 in the CSF. Also the P_{CO_2} rose to a level less t when Na_2CO_3 was used, increasing at the same rate in the blood and C

TABLE 5
INFUSION OF SODIUM CARBONATE* IN THREE DOGS DURING ONE-HALF HOUR OF APNEIC OXYGENATION

Dog (No.)	Wt. (kg.)	Time	Arterial blood					CSF					
			CO_2 (mM/l.)	pH	pCO_2 (mm. Hg)	HCO_3^- (mM/l.)	Mean pressure (mm. Hg)	CO_2 (mM/l.)	pH	pCO_2 (mm. Hg)	HCO_3^- (mM/l.)	M pre (n H
128	17	0′	21.99	7.34	39.0	20.82	137	23.34	7.35	42.2	22.02	
		30′	61.45	7.34	109.4	58.16	215	28.74	7.04	100.5	25.60	2.
		90′	39.46	7.40	62.6	37.58	162	32.51	7.26	70.8	30.30	
129	15	0′	19.57	7.39	31.7	18.62	173	22.68	7.45	33.2	21.64	
		30′	57.62	7.49	76.7	57.31	189	26.36	7.21	64.9	24.34	1
		90′	34.68	7.45	48.7	33.22	162	27.32	7.34	50.2	25.75	
1219	17	0′	20.80	7.38	34.1	19.77	133	21.90	7.32	42.1	20.59	1
		30′	50.64	7.25	110.2	47.32	150	27.63	7.03	99.1	24.54	1
		90′	31.59	7.43	46.4	30.19	133	27.94	7.29	57.3	26.15	
Av.		0′	20.79	7.37	34.9	19.74	148	22.64	7.37	39.2	21.42	
		30′	56.57	7.36	98.8	54.26	186	27.58	7.09	88.2	24.83	1
		90′	35.24	7.43	52.6	33.66	152	29.26	7.30	59.4	27.40	

* One ml./kg./min. of 0.2 M Na_2CO_3.
Key: 0′ = control (M.V.); 30′ = after 30′ apneic oxygenation; 90′ = 60′ after en apnea (M.V.).

The blood pH was kept almost constant, a slight increase being observed in three experiments, whereas the pH in the CSF decreased by an average of pH U., due to the not fully compensated rise in free carbonic acid.

One hour of mechanical ventilation brought the arterial acid-base st back to preapneic values in the dogs that had not received buffer. In the that had received Na_2CO_3 or THAM, only about 50 per cent of the rise arterial HCO_3^- had been eliminated during the first hour following infus The HCO_3^- therefore continued to rise slightly in the CSF during this per

The average rise in mean arterial pressure was 28 mm. Hg and in CSF p sure 152 mm. H_2O after one-half hour of apneic oxygenation with uncom sated respiratory acidosis (TABLE 4). The infusion of Na_2CO_3 did not elimi these rises in arterial and CSF pressures (TABLE 5). When the hyperca acidosis was buffered by THAM, the pressures were almost stable (TABLE

Discussion

ahas *et al.*[8] earlier have reported that intravenous infusion of 0.3 *M* THAM e rate of 1.1 ml./kg./min. kept the *p*H constant in dogs that were main- ed in apneic oxygenation for 60 minutes. Calculated on the basis of com- neutralization of all carbon dioxide produced in the body, this is an almost hiometric amount of THAM. As could be expected, one-half of that unt administered during constant ventilation caused a drop in $PaCO_2$ in all , and an increase in HCO_3^-, resulting in an alkalosis in blood as well as in .

TABLE 6

SION OF THAM* IN THREE DOGS DURING ONE-HALF HOUR OF APNEIC OXYGENATION

g .)	Weight (kg.)	Time	Arterial blood					CSF				
			CO_2 (m*M*/l.)	*p*H	*p*CO_2 (mm. Hg)	HCO_3^- (m*M*/l.)	Mean pressure (mm. Hg)	CO_2 (Mm/l.)	*p*H	*p*CO_2 (mm. Hg)	HCO_3^- (m*M*/l.)	Mean pressure (mm. H_2O)
	10	0′	17.82	7.41	27.4	17.00	135	20.26	7.36	35.9	19.14	67
		30′	42.51	7.43	62.0	40.64	148	24.37	7.20	60.4	22.49	94
		90′	35.62	7.31	67.4	33.59	189	27.31	7.19	68.8	25.16	40
(a)	14	0′	19.32	7.43	28.2	18.47	162	20.44	7.36	35.8	19.32	70
		30′	43.20	7.50	54.3	41.57	179	23.33	7.29	47.7	21.84	80
		90′	26.53	7.52	31.9	25.57	195	23.17	7.39	38.2	21.98	40
(b)	13.5	0′	19.64	7.40	31.0	18.71	158	20.60	7.34	37.9	19.42	60
		30′	45.90	7.49	59.0	44.12	160	26.56	7.24	60.3	24.78	70
		90′	30.66	7.38	50.4	29.14	162	26.54	7.26	58.1	24.73	50
		0′	18.93	7.41	28.9	18.06	152	20.43	7.35	36.5	19.29	66
		30′	43.87	7.47	58.4	42.11	162	24.75	7.25	56.1	23.04	81
		90′	30.94	7.40	49.9	29.43	182	25.67	7.28	55.0	23.96	43

One ml./kg./min. of 0.4 *M* THAM.

y: 0′ = control (M.V.); 30′ = after 30′ of apneic oxygenation; 90′ = 60′ after end of (M.V.).

equimolar amount of $NaHCO_3$ given during constant ventilation, which ented elimination of the CO_2 load, resulted in an increase in $PaCO_2$ as well HCO_3^-. However, the rise in HCO_3^- was larger, causing the ratio HCO_3^-/ D_3 to increase. As equilibration of dissolved CO_2 is a more rapid process that of H^+ and HCO_3^- ions, the alkalosis in the blood was accompanied by idosis in the CSF. This again demonstrates the importance of an intact function when $NaHCO_3$ is used to correct acidotic conditions.

hen Na_2CO_3 was used, there was no increase in $PaCO_2$ under these condi- of constant volume ventilation. The marked increase of HCO_3^- in the l was not accompanied by a similar increase in the CSF, and therefore no ine shift in the CSF occurred.

the experiments with a concomitant acid load, infusions of 0.4 *M* M and of 0.2 *M* Na_2CO_3 were used at the same rate, in amounts that are ned to be equivalent. The amount of THAM was slightly higher than the amount used earlier to compensate total retention of carbon dioxide in d The slight rise in blood pH during one-half hour of apneic oxygenation, w 0.4 M THAM was infused at a rate of 1 ml./kg./min., is thus in agreement earlier observations. The amount of 0.2 M Na_2CO_3 infused at the same also resulted in a slight overcompensation of the respiratory acidosis. Th was, however, a greater rise in $PaCO_2$, as well as in HCO_3^- with Na_2CO_3 t with THAM. As HCO_3^- diffuses more slowly than free CO_2, this resulted greater dissociation between blood and CSF pHs when Na_2CO_3 was used t when THAM was used to buffer the acid load. It may be of special inte that the HCO_3^- increase in the CSF was on an average slightly higher du THAM infusion than during infusion of Na_2CO_3, in spite of the much hi blood-to-CSF HCO_3^- gradient in the latter experiments. This may indi diffusion of THAM into the CSF already within the first one-half hou administration and buffering of CO_2 within that fluid compartment.

The much smaller changes in blood and CSF pressures when THAM ins of Na_2CO_3 was used to buffer the respiratory acidosis during apneic oxyg tion may also indicate a difference in distribution of the two buffers. As blood pH was kept normal with both buffers, one may advance the hypoth that the stimulation of the sympathicoadrenal system in hypercapnic aci is connected with intracellular pH changes, and that THAM may act a intracellular buffer. Preliminary experiments[9] with C^{14} tagged THAM indi that THAM distributes in 65 to 80 per cent of the total body water with to 6 hours.

Summary and Conclusions

The acid-base relationship between blood and CSF following rapidly indu changes in the arterial bicarbonate/carbonic acid ratio was investigated in d Intravenous infusion of $NaHCO_3$ during constant-volume mechanical ven tion changes the pH in blood and CSF in different directions, whereas infu of THAM results in an alkaline shift in both compartments. This differ is due to the more rapid equilibration of dissolved CO_2 than of HCO_3^-.

When Na_2CO_3 and THAM are used to keep arterial pH constant durin vere respiratory acidosis, there is an acid shift in CSF, which, however, is pronounced with Na_2CO_3 than with THAM.

The use of THAM to buffer respiratory acidosis eliminates the rise in b and CSF pressures, whereas Na_2CO_3, in spite of keeping the blood pH const does not have this effect. This difference may indicate that the stimulatio the sympathicoadrenal system in hypercapnic acidosis is connected with in cellular pH changes, and that THAM acts as an intracellular buffer.

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EFFECTS OF 2-AMINO-2-HYDROXYMETHYL-1,3-PROPANE-DIOL, pCO_2, AND pH ON PLASMA PROTEIN BINDING OF THE THYROID HORMONES*

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Introduction

The problem of the binding and transport of the thyroid hormones—t roxine and triiodothyronine—by specific plasma proteins has been the sub of recent intensive investigation. For the purpose of this discussion, we f summarize briefly current concepts in this area based on the work of ma investigators and reviewed in several recent reports.[1-7] With this as a fra work of reference, we shall present certain of our findings, some of which h already been published,[8,9] concerning the effects on thyroid hormone bind of Tris buffer, carbon dioxide tension, and pH.

Early workers[10-12] employed chiefly paper electrophoretic techniques us Veronal buffer at a pH of 8.6. In extensive studies based on this method was found that more than 90 per cent of physiological levels of thyroxine (was bound in a highly selective fashion to a trace interalpha globulin prot in plasma, designated the thyroxine-binding protein or TBP. Small amou of T4 were also found in the albumin fraction. Triiodothyronine (T3) migra principally to the same general area, but the binding was much weaker and selective.

Many significant physiological correlations were made, based on such stud For example, it was found that the percentage of T4 bound in the TBP a was decreased in thyrotoxicosis and increased in myxedema. It was fou further that there was a marked increase in T4 binding in pregnancy and lowing estrogen administration, as well as a marked decreased binding in nephrotic syndrome. The weaker T3 binding in plasma was proposed a major explanation for its more rapid onset of action and greater potency.

Studies with Tris Buffer

More recently, however, by the use of other buffers,[13-18] it has been fo that there may be an additional specific binding protein for T4, but not for which migrates ahead of albumin and is called thyroxine-binding prealbu protein (TBPA). We "stumbled upon" this phenomenon when we used instead of Veronal buffer, based on the finding that Tris buffer results in electrophoretic separation of several additional plasma protein fractions. example, FIGURE 1 depicts the pattern of radioactivity (lower section) a starch-gel electrophoresis[19] in Tris buffer, pH 8.6, of normal human serum which I-131 T4 had been added (upper section). It can be seen that, in a

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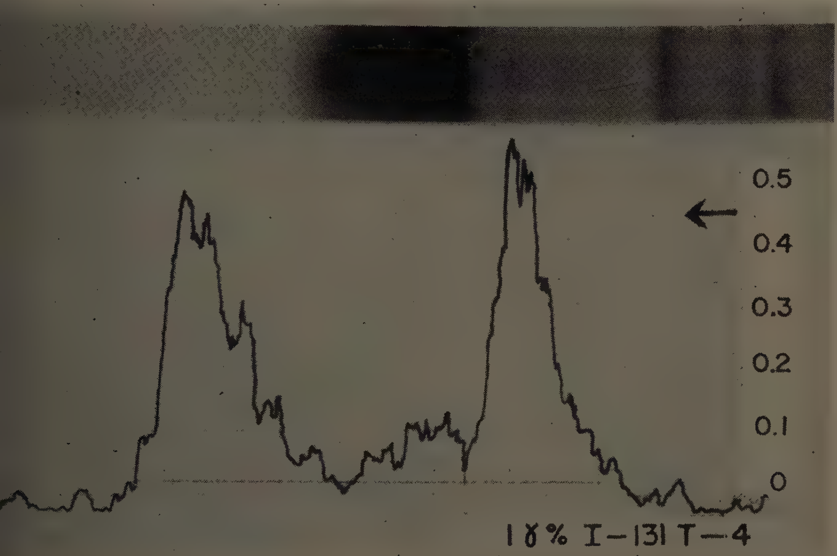
to a large peak in the interalpha globulin area and a small peak in the al-
hin area, there is a large peak migrating ahead of the broad albumin band
ere a faint protein stain can be detected on the original preparation. Similar
lts have been obtained by others[13-18] using Tris and other buffers at *p*H
Such results have forced a re-evaluation of the former conclusions: a
valuation that is still in process.[5-7]

or example, as seen in FIGURE 2, we electrophoresed by the usual standard
nniques normal serum labeled with T4 on paper in Veronal buffer at *p*H



IGURE 1. Starch-gel electrophoresis (*upper strip*) of normal human serum, exogenously
ed by addition of 1 μg. I-131 T4 (migration from right to left); Tris-maleate buffer, *p*H
Lower section depicts correlative scanning of radioactivity. From right to left: (1)
alpha globulin area, (2) proximal albumin area, (3) prealbumin area (faint protein stain,
le on original preparation, not reproduced in photography). Reproduced by permission
Endocrinology (Hamolsky *et al.*, 1961).

then cut out the two major radioactive peaks (TBP and albumin), in-
ed each strip of paper in a starch gel and re-electrophoresed each segment,
time in Tris or borate buffer. We found that the TBP single peak was
lved routinely into at least two and occasionally three separable peaks of
ein-containing radioactivity. When we re-electrophoresed the albumin
nent, we found that the bulk of the radioactivity was separate from the
mass of stainable albumin and migrated behind it in a sharp peak where
rotein could be seen. Thus it may be seen that there is a further hetero-
ity of the specific binding protein elements depending upon the technical
ess utilized; this necessitates caution in attempting physiological interpre-
n of the significance of altered binding capacity in various diseased states.
our continuing study of the individual binding proteins, we eluted the

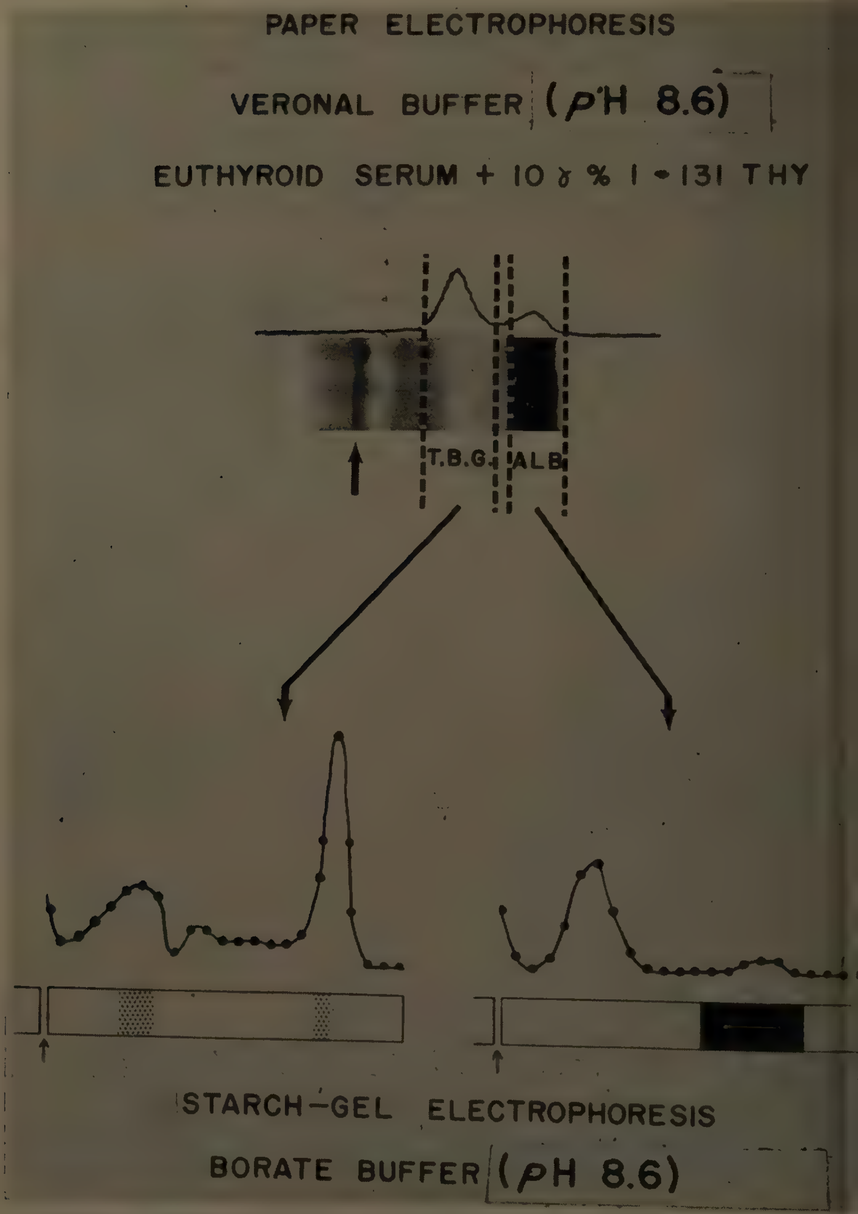


FIGURE 2. Combined paper and starch-gel electrophoresis of normal serum, exogenou labeled by addition of 10 μg. I-131 T4. In each of 3 major sections, the lower portion rep sents distribution of protein, and the upper portion the correlative radioactive scan. T section: paper electrophoresis of whole serum—Veronal buffer—pH 8.6. Bottom sectio left: re-electrophoresis of paper TBP segment (starch gel–borate buffer, pH 8.6). Bott section, right: reelectrophoresis of paper albumin segment (starch gel–borate buffer, pH 8. Reproduced by permission from *Endocrinology* (Hamolsky *et al.*, 1961).

bumin segment from the Tris starch gel and then studied its binding city by its ability to decrease the uptake of I-131 T4 by washed red cells ended in saline. As seen in TABLE 1, when an aliquot of the prealbumin e was added to the red-cell suspension, the uptake of T4 (expressed as cent/100 hematocrit) was decreased from 134 to 55. To our surprise, n the eluate was dialyzed against water or saline and then retested, its

TABLE 1

FFECT OF PREALBUMIN ON I-131-THY UPTAKE BY WASHED RED BLOOD CELLS

Test system	Uptake (per 100 hematocrit) (%)
ontrol (RBC in saline)	134
.2 ml. prealbumin*	
a) Eluate before dialysis	55
b) After dialysis against saline	136
.2 ml. prealbumin (2-B) after redialysis against	
0.05 *M* Tris buffer	116
0.10 *M* Tris buffer	109
0.30 *M* Tris buffer	79
0.50 *M* Tris buffer	53
0.60 *M* Tris buffer	43

fect of TBPA, eluted from starch gel (electrophoresis of normal serum-Tris buffer, *p*H n the uptake of I-131 T4 by human erythrocytes, washed ×3 in saline, then resuspended ine. Effect of dialysis (16 to 18 hours) of eluate against saline. Effect of redialysis her 18 hours) of saline dialyzed eluate against varying concentrations of Tris buffer. oduced by permission from *Endocrinology* (Hamolsky *et al.*, 1960).

Eluted from starch gel after electrophoresis of euthyroid serum (0.5 *M* Tris buffer).

TABLE 2

EFFECT OF TRIS BUFFER ON *IN VITRO* RBC UPTAKE OF I-131-THY FROM WHOLE BLOOD (2 HOURS)*

Molarity of added Tris	Uptake/100 hematocrit (%)
0 (control blood)	2.19
0.06	1.78
0.125	1.53
0.25	1.10
0.50	1.09

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ng capacity was gone. After a lengthy and fruitless search for the bind- nolecule outside the bag, we turned back to the dialyzed bag and found if we redialyzed the eluate against Tris buffer we could regain the binding city, which was then proportional to the molarity of the Tris buffer used edialysis. Thus redialysis against increasing amounts of Tris buffer d increased binding by the eluate and, therefore, a progressively decreased ell uptake. When increasing amounts of Tris were added directly to e blood (TABLE 2), the *in vitro* uptake of T4 by the red cell from plasma similarly progressively decreased. This appears to be due to an increased plasma binding in Tris, as the addition of Tris to red cells in saline alone not decrease the red cell uptake of T4. We then studied[8,9] the effect of on the dialysis of T4 from human serum albumin inside a dialysis bag, in plasma solution outside the bag (TABLE 3). As increasing amounts of were added to the saline-plasma mixture outside the bag that containe bound to albumin, there was a progressive increase in the amount of T4 passed from the bag into the plasma. Again Tris increased the binding tency of plasma against a standardized amount of albumin. The effe

TABLE 3

EFFECT OF TRIS BUFFER IN PLASMA ON TRANSFER OF I-131-THY FROM HUMAN SERUM ALBUMIN ACROSS DIALYSIS MEMBRANE*

Composition of medium surrounding dialysis bag	I-131-THY transferred to p (%)	
	3 hr.	22 hr
1 ml. plasma + 9 ml. saline	15.8	42.2
1 ml. plasma + 9 ml. saline + 0.05 *M* Tris buffer	16.2	45.1
1 ml. plasma + 9 ml. saline + 0.2 *M* Tris buffer	21.7	61.3
1 ml. plasma + 9 ml. saline + 0.5 *M* Tris buffer	23.5	71.0

* Bag contents = 2 mg. human serum albumin, 0.012 μg. I-131-THY, 1.0 ml. saline
Reproduced by permission of *Endocrinology* (Hamolsky *et al.*, 1960).

TABLE 4

EFFECT OF VARIOUS BUFFERS ON TRANSFER OF I-131-THY FROM HUMAN SERUM ALBUMIN ACROSS DIALYSIS MEMBRANE*

Composition of medium surrounding dialysis bag	I-131-THY transferred (%)	
	4 hr.	22 hr
10 ml. Krebs-Henseleit buffer (*p*H = 7.4)	11.7	20.4
10 ml. 0.2 *M* Tris buffer (*p*H = 8.6)	11.8	20.0
10 ml. 0.2 *M* phosphate buffer (*p*H = 8.7)	10.5	19.3
10 ml. 0.2 *M* Veronal buffer (*p*H = 8.6)	38.2	60.6

* Bag contents = 2 mg. human serum albumin; 0.012 μg. I-131-THY, 1 ml. saline.
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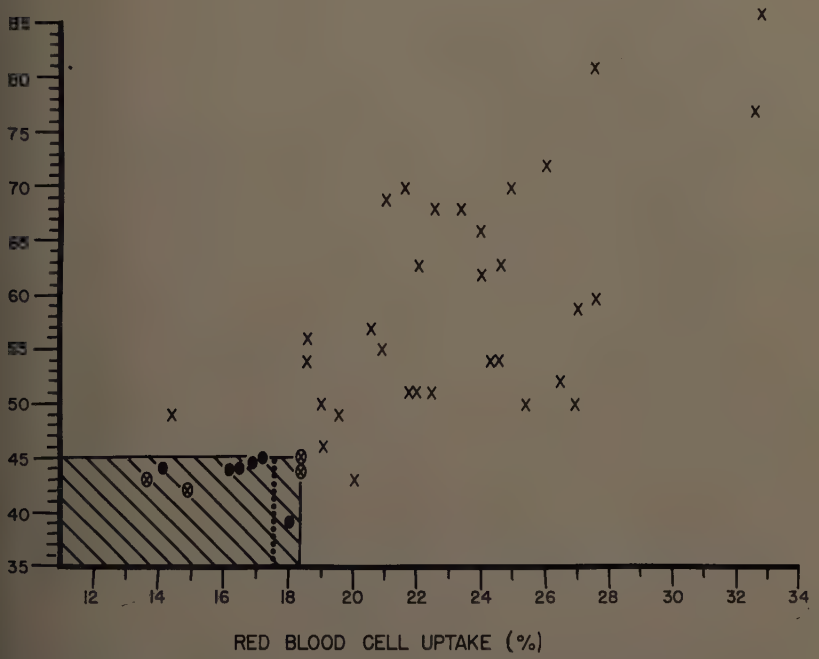
various buffers without plasma on the dialysis of T4 from a standard hu serum albumin mixture was then studied (TABLE 4). It may be seen whereas Krebs-Henseleit, Tris, and phosphate buffers, outside the bag, effe essentially the same amount of T4 dialysis, Veronal buffer, which had used almost exclusively in early electrophoretic studies, resulted in a mark increased dissociation of T4 from the albumin and dialysis across the m brane. Once again the importance of technical factors in assessing the and relative protein binding of thyroid hormone is underscored.

Studies of the Effects of pCO_2

In addition to electrophoretic studies of hormone binding, the distribu of radioactive thyroid hormone between plasma protein on the one hand ous tissues on the other has been studied. We have previously reported[20,21] he development and application of an *in vitro* method that measures the poration of I-131 T3 by red blood cells (RBC) from whole blood as an x of thyroid function. On the basis of more than 9000 determinations in ents, we have found that the 2-hour *in vitro* RBC uptake of I-131 T3 from e blood ranges from 11 to 17 per cent in 94 to 95 per cent of euthyroid les; from 11.5 to 18.5 per cent in euthyroid males. Uptakes are elevated per cent of hyperthyroid patients and decreased in 98 per cent of hypo- oid subjects.



GURE 3. Correlation of *in vitro* RBC uptake of I-131 T3 from whole blood with pCO$_2$ percapneic patients (see text for details). Reproduced by permission from *Endocrinology* olsky *et al.*, 1961).

the course of our study of factors responsible for abnormal RBC uptakes herwise euthyroid subjects, the effect of Tris in lowering uptakes and its n CO$_2$ buffering capacity focused our attention on hypercapneic patients. GURE 3 are plotted 32 red cell uptakes (abscissa) in 22 consecutive pa- s with CO$_2$ retention (ordinate). Nineteen had chronic pulmonary em- ema, 2 had kyphoscoliosis, and 1 had marked hypoventilation following rovascular accident. The hatched areas indicate the normal values in boratories. The dotted vertical line represents the upper limit of normal emales, the solid line for males. A highly significant correlation between and red cell uptake is observed with an r value of 0.92. The solid cir- epresent normal uptakes in 6 patients with severe dyspnea from pulmo- emphysema but with normal pCO$_2$ values. The circled crosses represent

4 patients with previously elevated values whose uptake fell to normal pCO$_2$ fell in response to therapy. In FIGURE 4 are plotted the simultane red cell uptakes (circled dots) and pCO$_2$ values (numbers in parentheses) i young patient with severe kyphoscoliosis. Both values were elevated hospital entry and with a severe exacerbation of the patient's disease reach very high values with a gradual return to normal with effective therapy. T elevated RBC uptake depends upon an abnormality in the plasma binding thyroid hormones as, in repeated "criss-cross" studies, red blood cells fro normal subject have elevated uptakes in hypercapneic plasma whereas c

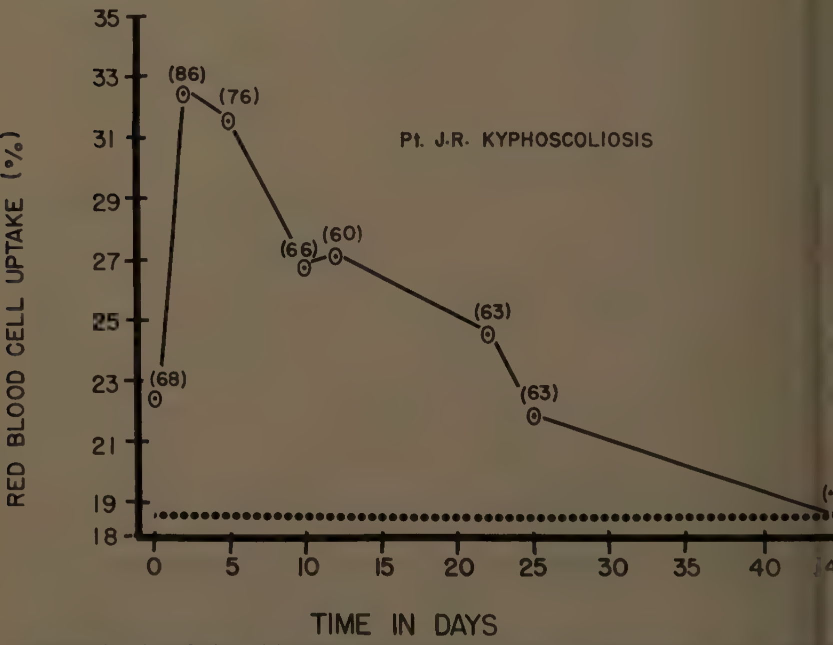


FIGURE 4. Correlation of *in vitro* RBC uptake of I-131 T3 from whole blood (*circled* and pCO$_2$ (*in parentheses*) in patient with kyphoscoliosis and exacerbation of hypercapne

from a hypercapneic patient have normal uptakes in normal plasma. fact that this decreased plasma binding, hence increased tissue incorporati of thyroid hormone may be of some physiological significance is suggested the finding that when I-131 T4 was infused *in vivo* into 8 of these hyperc neic patients the half-times of disappearance of the infused thyroxine ran from 4.2 to 5.8 days, averaging 5.1 days. This is a much more rapid tu over than in normal patients in whom half-times range from 6.6 to 7.8 d with an average of 7.2 days.

We have been able to reproduce some of these phenomena experiment The red cell uptake of T4 or T3 is decreased up to 50 per cent *in vitro* if pCO$_2$ is permitted to fall as by incubating in an open flask. The uptake be returned to normal simply by restoppering the flask and continuing the or by maintaining a normal pCO$_2$ during the run by passing CO$_2$ into the ppered flask. Red cell uptake can be increased above normal by bubbling CO_2 under pressure. It is noteworthy, however, that to date we have not n able to obtain uptake values as high as those obtained in our hypercap- patients. Normal uptakes, once attained, decrease if the flask is then toppered and the run continued. Similarly the dialysis of T4 or T3 from sma to a buffer medium can be decreased or increased respectively by de- asing or increasing the surrounding $p\mathrm{CO}_2$. The increased dialysis is ob- ved following the increased $p\mathrm{CO}_2$ despite the maintenance of *p*H within a row range by the concomitant addition of bicarbonate ion.

Studies of the Effects of pH

)ther studies, however, have revealed a separate role of *p*H in affecting mone binding. The dialysis of T4 and T3 from plasma into various buffers different *p*Hs has been determined. FIGURE 5 depicts in composite fashion percentage of T4 and T3 dialyzed from whole plasma and various separate ding components into Tris buffer at different *p*Hs. The binding of T4 by ole plasma is progressively increased (that is, dialysis is decreased) from d pHs, through 7.4 to a peak binding at about *p*H 8.0 to 8.2, followed by a rp decrease in the binding. The binding of T3 in both toxic and euthyroid sma is similarly less below 7.4, increasing to a peak at about 8.0 but show- either no decrease or only a slight decrease above *p*H 8.2. We have at- pted to characterize the *p*H effect on the separate binding proteins to the ent that we have been able to prepare them in relatively purified form. example, in the next section of FIGURE 5, there is plotted the binding of by a highly purified, chemically prepared fraction of TBP from fraction of serum showing an increased binding from *p*H 4.2 to 7.4, followed by a rease. In contrast, in the next section of FIGURE 5, the binding of T3 by same TBP fraction is essentially constant over a wide *p*H range. The t section demonstrates the binding of T4 by human serum albumin showing rogressive increased binding from 7.4 to 8.4, remaining essentially constant reafter. The next section demonstrates that the effect of *p*H on binding T3 by human serum albumin is essentially the same as that for T4. The ding of T4 by prealbumin obtained by elution from a starch gel increases rply from acid *p*Hs towards the more alkaline side. It is particularly note- thy that the binding of T4 by prealbumin at a physiological *p*H of 7.4 is y weak (dialysis carried out for only 3½ hours in contrast to the 18 hours essary for significant dialysis of T4 from plasma, TBP, and albumin). A ilar point has been made by Myant and Osoris,[18] who found on electro- resis that, whereas thyroxine is bound rather strongly to TBPA at *p*H 8.6, re is little or no binding of thyroxine to the prealbumin area at *p*H 7.4. h results raise a significant question concerning the physiological signifi- ce of binding by the prealbumin fraction. Thus it may be concluded that binding of T4 and T3 by human serum plasma components is affected erimentally by alterations in *p*H and that the resultant over-all binding be considered in terms of different effects of *p*H on different binding en- s.

Ve have recently attempted to delineate a possible role of *in vivo* *p*H altera-

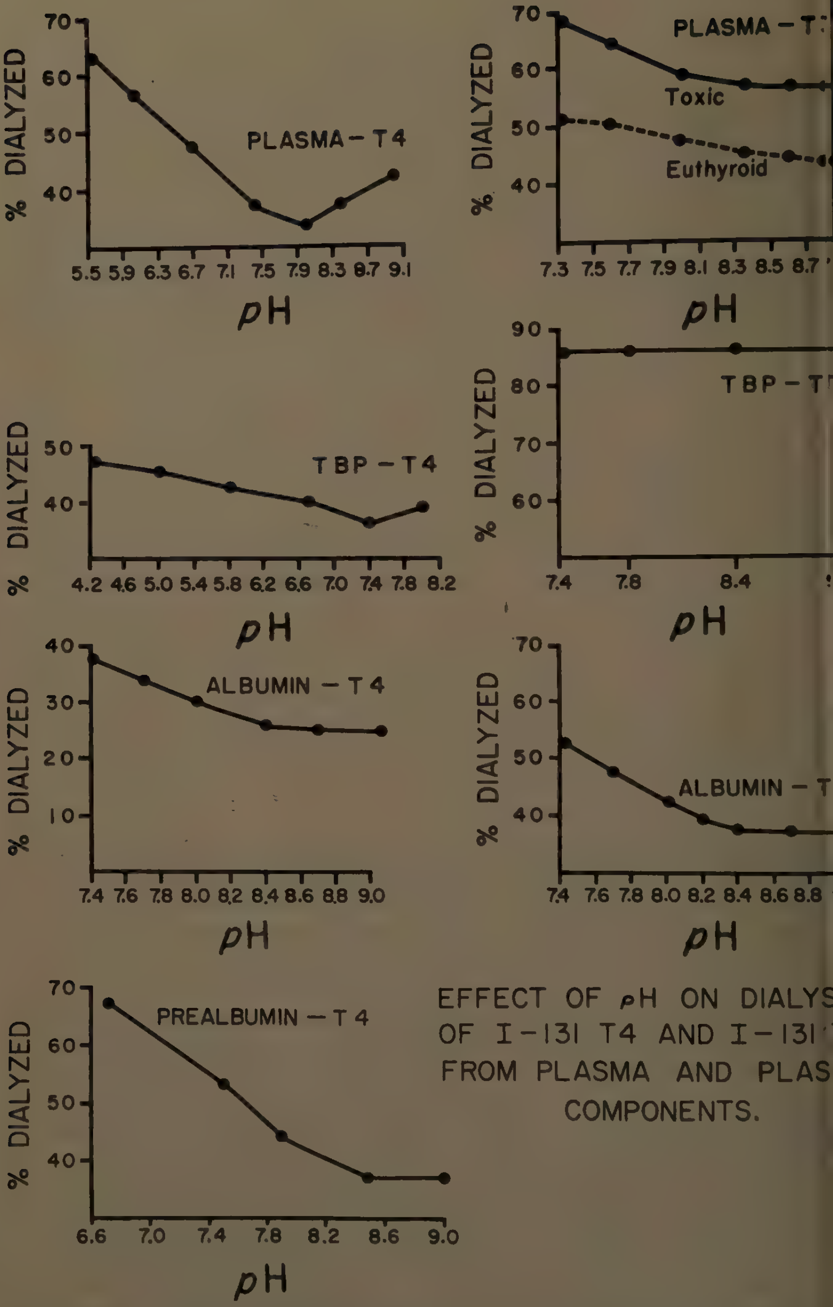


Figure 5. Composite representation of dialysis of T4 (*left side*) and T3 (*right side*)
whole plasma and separate binding components at varying *p*Hs (Tris buffer). Durati
dialysis: for T4 from plasma, TBP, and albumin—16 to 18 hours; T4 from TBPA—3½ h
T3 from plasma, TBP, and albumin—3½ hours. Bag contents: plasma, 0.02 ml. plasma
ml. saline, containing I-131 T4 or T3 to increase total PBI by 5 μg. per cent; TBP, 0.
highly purified preparation from fraction IV of serum in 1 ml. saline, with T4 or T3 t
crease PBI by 5 μg. per cent; albumin, 4 mg. albumin in 1 ml. saline with T4 or T3 add
PBI increase of 5 μg. per cent; TBPA 0.2 ml. of eluate from starch gel (normal human seru
I-131 T4 to PBI increase of 1 μg. per cent. Surrounding medium = 7 ml. of Tris buf
varying *p*H, 0.05 *M*.

ns in thyroxine binding and transport. For that purpose we have used the
g and have produced various alterations in pCO_2 and pH by temporary
riods of apnea, varying oxygenation, and compensatory infusions of Tris
bicarbonate buffer. It would appear from our early results that in the
ort-term, acute, *in vivo* experiments, the pH plays a major role in determin-
g the binding of T3 and T4 in dog blood. As seen in FIGURE 6, despite
arked alterations in pCO_2 in these experiments, the red cell uptake appeared
be determined by the pH of the blood at the time it was drawn: increasing
acid pH, decreasing at alkaline pH. The curve suggests a sharp point of
lection near pH 7.4. We are now engaged in experimental studies attempt-
g to define further the possible physiological role of pH alterations in thy-
ine metabolism.

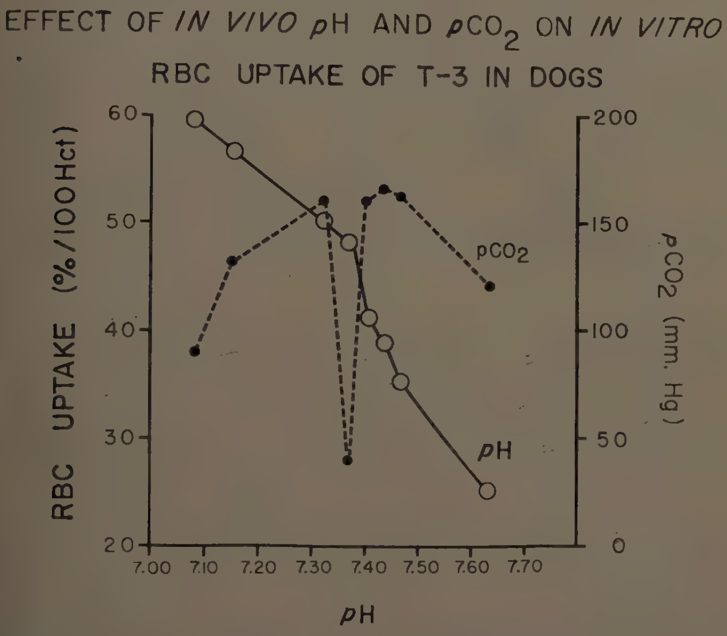


FIGURE 6. Effect of *in vivo* alteration of pH and pCO_2 levels in dogs by apneic oxygena-
n with infusions of Tris or bicarbonate buffer on *in vitro* RBC uptakes of I-131 T3 from
g whole blood. pH versus RBC uptake = open circles connected by solid lines; pCO_2
sus RBC uptake = solid circles connected by broken lines; Hct = hematocrit.

Summary

We have reviewed briefly the concept of binding of thyroid hormones by
ecific plasma proteins. Results indicate that interpretations of binding
enomena in physiological terms must involve consideration of the technical
tors involved. Heterogeneity of the binding moieties is suggested. In
r experiments, Tris buffer appeared to play a significant role in increasing
al thyroxine binding by plasma protein and also in altering the relative dis-
bution of thyroid hormones between the various proteins. Patients with

hypercapnea demonstrated decreased plasma binding and an increa thyroxine turnover. Hormone binding was decreased by experimental percapnea, increased by hypocapnea. The *p*H of the surrounding medi affected hormone binding; binding by plasma was decreased at acid *p*Hs, creased at alkaline *p*Hs. The capacity of the total plasma protein to bi T4 or T3 at different pHs may be considered in terms of a balance of separate effects of *p*H alteration on the individual binding moieties. In sho term *in vivo* experiments in dogs, alteration of *p*H affected *in vitro* hormo binding. The possible physiological significance of alterations of such fact as $p\text{CO}_2$ and *p*H in total thyroidal economy warrants further investigation.

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INTRACELLULAR ACID-BASE RELATIONS AND INTRACELLULAR BUFFERS

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The classical work of Henderson and Hasselbalch established firmly the oretical basis of defining acid-base relationships of plasma and extracellular id. Methods based on this theoretical foundation have been widely used by logists to develop present knowledge of this area. Implicit in an interpreta- n of the results of investigations of extracellular acid-base balance has been assumption that a given change of extracellular pH is accompanied by a ilar change both in direction and magnitude of intracellular pH. During past 40 years, however, a large amount of evidence has accumulated indi- ing that hydrogen ion concentrations across cell membranes do not obey Donnan relationship and that, under many circumstances, intracellular may be independent of extracellular pH.

n this basis, any evaluation of the effect of a given substance upon acid- se metabolism should include an estimate of the effect of the substance on racellular pH.

uch evaluation is obviously a complex matter. Intracellular water is not a mogeneous unit, and there is undoubtedly considerable variation in pH from to cell as well as nonuniformity of H^+ ion concentration within individual ls. Despite these variations, it is theoretically possible to develop a tech- ue for approximating a value that reflects the mean or average H^+ concen- tion inside cells throughout the body.* Such a technique has the dis- vantage of providing no exact information about regional or single-cell pH ues but does have the advantage of providing data in the intact animal and ther, of being a reflection of pH changes in the great bulk of cells.

he method to be described is based on work originally performed by Wad- and Butler, who utilized the same general approach for the measurement the internal pH of muscle cells. It uses the weak acid 5,5-dimethyl-2,4- zolidinedione (DMO) as a pH indicator. The theoretical basis of the thod is as follows:

n extracellular water un-ionized DMO (HDMO) dissociates to form H^+ and ionized DMO^-.

$$\mathrm{HDMO}_e \rightleftharpoons H_e^+ + \mathrm{DMO}_e^-$$

equilibrium the quantitative relationship between the ionized and un- ized portions is expressed by the mass action law

$$(\mathrm{H}^+)_e \cdot (\mathrm{DMO}^-)_e = \mathrm{K}_{a_e} \cdot (\mathrm{HDMO})_e \,.$$

As a matter of fact, using a weak acid as the indicator system will give rise to a "mean" e that is nearer to the regions of high pH than to regions of low pH.

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A similar relationship exists in intracellular water.

$$(H^+)i\ (DMO^-)i = K_{a_i}\ (HDMO)i$$

As a close approximation, it may be assumed that the dissociation consta for this reaction in extra- and intracellular water are equal (that is, $K_{a,}$ K_{a_i}). Furthermore, it seems likely that most cell membranes are freely per able to the un-ionized form (HDMO) but are relatively impermeable to ionized form (DMO^-). If these assumptions are true, then at equilibrium activities of the un-ionized portion (HDMO) in extracellular and intracell water should be equal (that is, $(HDMO)_e = (HDMO)_i$) and the relations reduces to the following:

$$(H^+)_e \cdot (DMO^-)_e = (H^+)_i \cdot (DMO^-)i$$

namely that the ratio of extracellular fluid hydrogen ion concentration intracellular fluid hydrogen ion concentration is *inversely* equal to the rati concentration of ionized DMO in the two compartments. In brief, the hy gen ion concentration of extracellular fluid may be experimentally determi as the pH of plasma; the concentration of ionized DMO in extracellular f may be calculated from the pH, total DMO concentration (that is, (DMO^-) $(HDMO)_e$) in extracellular fluid, and the pK of this equilibrium; the aver concentration of ionized DMO in intracellular fluid can be determined f suitable experimental data. Hence, the mean hydrogen ion concentratio intracellular fluid may be calculated.

The logarithmic form of the mass action law is the most convenient, and following derivation provides an expression for the concentration of HD in terms of extracellular pH, extracellular total DMO concentration and $pK'a$ of this reaction.

$$pH_e = pK'a + \log \frac{(\text{total DMO})e\ -\ (\text{HDM}}{(\text{HDMO})e}$$

$$(pH_e - pK'a) = \log \frac{(\text{total DMO})e}{(\text{HDMO})e} - 1$$

$$(10^{pH_e - pK'a} + 1)\ (\text{HDMO})_e = (\text{total DMO})_e$$

$$(\text{HDMO})e = (\text{HDMO})i = \frac{(\text{total DMO})e}{10^{pH_e - pK'a} + 1}$$

Restating the mass action equation for the pH of intracellular water, following expression is obtained:

$$(pH)i = pK'a + \frac{(\text{total DMO})i - (\text{HDMO})i}{(\text{HDMO})i}$$

$$= pK'a + \log \frac{(\text{total DMO})i\ (10^{pH_e - pK'a} + 1)\ -\ (\text{total DM}}{(\text{total DMO})e}$$

The value of $pK'a$ as given by Waddell and Butler is 6.13 at 37° C. only experimental parameter that remains to be determined is the concen on of total DMO in intracellular water. This value may be obtained from easurements of total body water, extracellular water, extracellular total MO concentration, and a knowledge of the total mass of DMO injected. leasurements have been performed on three species; man, the spiny dogfish, nd the dog. This paper will be confined to a discussion of a few of the results btained in the dog.

Of the theoretical and practical limitations of the technique, only two will e discussed.

First, it should be pointed out that, conceptually, it is difficult to define xactly what is meant by extracellular fluid. Should this term include spinal uid, aqueous humor, gastrointestinal water, and bone water? Since extrallular water is estimated as inulin space in the dog, what is actually measured y the described technique is the *p*H of the "noninulin space." That such a pace has a physiologic meaning must be assumed.

TABLE 1

REPRODUCIBILITY OF INTRACELLULAR *P*H MEASUREMENTS IN SEVEN DOGS

*p*H*e*		*p*H*i*	
3 hr.	4 hr.	3 hr.	4 hr.
7.50	7.48	7.06	7.03

TABLE 2

MEAN WHOLE BODY INTRACELLULAR *P*H DETERMINATIONS ON 38 DOGS UNDER CONTROL CONDITIONS

*p*H*e*	7.45
*p*H*i*	7.08
*p*H*e* − *p*H*i*	0.37

A second important difficulty stems from the fact that each "intracellular" H determination requires four individual measurements. The total experiental error of the technique is, therefore, dependent upon the experimental rors of each of the determinations. A priori, it is difficult to predict within hat limits of accuracy intracellular *p*H determinations can be performed. me idea of the magnitude of error can be obtained by determining the reoducibility of the technique. Data on reproducibility were obtained by the termination of intracellular *p*H in 7 dogs at the end of a 3-hour equilibration riod and again at the end of a 4-hour period following the infusion of DMO. ese data are shown in TABLE 1. It may be seen that the variation of the erage plasma *p*H amounted to 0.02 *p*H units. The variation of the average tracellular *p*H at the same time amounted to 0.03 *p*H U. The technique, erefore, gives rise to values of quite acceptable reproducibility.

The relationship between extracellular and mean whole body intracellular H in the dog under control circumstances is shown in TABLE 2. Such deterinations have been performed on a total of 38 dogs with more than 50 individ-

ual determinations. These data show that the mean intracellular pH for t
group of animals averages 7.08 at a mean extracellular pH of 7.45. The
gradient amounts to 0.37 pH unit or, in other words, the hydrogen ion co
centration of intracellular water is more than twice that of extracellular flui
The relative acidity of whole body intracellular water as determined by t
DMO technique is consistent with measurements of the internal pH of mam
malian muscle made using glass electrodes, the CO_2-HCO_3^- system, and t
DMO system. Such studies (with one exception) have indicated a value f
muscle pH of approximately 7.0. This finding buttresses the theoretical ba
of the measurements reported here, since muscle tissue makes up a considera
part of the area whose pH is being measured by DMO. Although the mech
nisms that permit such a high pH gradient between plasma and cells are uncle
at present, it seems clear that conventional physiologic and biochemical a
proaches to acid-base problems based on the pH of extracellular fluid alo
will require modification.

As an illustration of this concept, the effects of the intravenous administr
tion of Tris (tris-hydroxymethylaminomethane), a primary amine, upon intr
cellular pH and intracellular buffers will be discussed. All studies we
performed in dogs whose renal vessels and ureters were ligated at the hilu
eliminating the possibility of renal excretion. It is useful to compare the effe
of Tris on extracellular and intracellular pH with the effect of bicarbonate a
ministration, since bicarbonate is the most important extracellular buffer a
is therapeutically the substance most widely used in the treatment of acidos
TABLE 3 summarizes the results of experiments in which intracellular pH w
measured before and after the intravenous infusion of approximately 1
mmole $NaHCO_3$. It may be seen that despite a 0.28-unit rise in pH of extr
cellular fluid, intracellular pH increased only 0.03 unit. The relative inabili
of administered bicarbonate to influence intracellular pH is likewise shown
the "apparent bicarbonate" concentrations in intracellular fluid. During t
control period, intracellular "apparent bicarbonate" concentration averag
10.86 mmole/l. After HCO_3 administration, it rose only 1.33 mmole/l. (to
value of 12.19 mmole/l.) despite a rise in extracellular concentration of 1
mmole/l. The slow equilibration of HCO_3^- from extracellular to intracellul
fluid generally is consistent with observations made on single cells by Jacob
isolated muscle by Wallace and Hastings[2] and by Waddell and Butler[3], on bra
cells by Tschirgi[4], and on spinal fluid by Robin[5] and his co-workers.

TABLE 3
EFFECT OF BICARBONATE ADMINISTRATION ON EXTRACELLULAR AND INTRACELLULAR
ACID-BASE RELATIONS

	Control	Experiments
pHe	7.30	7.58
pHi	7.04	7.07
pCO_2 (mm. Hg)	42	44
$(HCO_3^-)_e$ (mM/l.)	19.43	38.81
$(HCO_3^-)_i$ (mM/l.)	10.86	12.19

TABLE 4 summarizes the results of experiments in which intracellular pH s measured before and 60 min. after the intravenous infusion of approxi- tely 150 mmole of Tris. Unlike bicarbonate, Tris administration resulted a sharp and significant rise in intracellular pH (actually intracellular pH es somewhat more than extracellular pH). This rise of intracellular pH is ompanied by a marked increase in the "apparent bicarbonate" concentra- n of intracellular fluid. Ready cellular penetration of Tris, therefore, seems characterize the effect of this substance on acid-base balance. This interpre- ion is also in accord with the observation that it is more difficult to maintain increase of extracellular pH with Tris than with HCO_3^-, suggesting that racellular penetration and buffering are important in the metabolism of Tris l relatively unimportant in the case of HCO_3^-. It should be emphasized t ready penetration by Tris must not be interpreted to mean clinical superi- ty of Tris in the treatment of acidosis. The clinical usefulness of any buffer ends not only on its buffering capacity but on its therapeutic safety, on the age required to achieve a given effect, and on the presence and nature of e effects. Therapeutic evaluation of a buffer, therefore, depends on careful rmacologic and clinical studies.

TABLE 4

EFFECT OF TRIS ADMINISTRATION ON EXTRACELLULAR AND INTRACELLULAR ACID-BASE RELATIONS

	Control	Experimental
pHe	7.34	7.47
pHi	7.08	7.27
pCO$_2$ (mm. Hg)	39	48
$(HCO_3^-)_e$ (mM/l.)	18.60	30.60
$(HCO_3^-)_i$ (mM/l.)	11.57	21.43

tudies have also been performed in an attempt to characterize the kinetics which Tris leaves the extracellular compartment. Such studies were per- ed by administering C_{14} labeled Tris in tracer doses and simultaneously asuring (1) total body water, (2) extracellular fluid volume, (3) extracellular , (4) intracellular pH, and (5) radioactivity of Tris per millimeter of plasma. he proportion of Tris remaining in extracellular fluid as compared with s presumed to have penetrated into nonextracellular fluid as a function of e is shown in TABLE 5. It is apparent that Tris continues to be lost from acellular sites for periods up to at least 300 min. with no evidence of equilib- n being achieved. The fraction of Tris not present in extracellular fluid been designated as nonextracellular since it is not possible to distinguish ween intracellular buffering, transcellular buffering, and metabolic loss. the end of 300 min., approximately 28 per cent of Tris can be accounted for xtracellular fluid and 72 per cent in nonextracellular fluid.

he loss of Tris from extracellular fluid seems to follow a simple exponential n as shown in FIGURE 1 where Tris counts/ml. of plasma are plotted semi- rithmically against time. Seven such studies have been performed, and results in each have been entirely similar.

Such data are important in considering the possible mechanism of T penetration of cells. A priori, the most reasonable assumption would be consider that it takes place by nonionic diffusion. TABLE 6 shows data in cating that this may not be the case. Quantitatively, nonionic diffusion m

TABLE 5

PERCENTAGE OF C_{14} LABELED TRIS IN ECF AND NON-ECF FROM 60 TO 300 MINUTE

	60 min.		120 min.		180 min.		240 min.		300 min.	
	ECF Tris (%)	Non-ECF Tris (%)	ECF Tris (%)	Non-ECF Tris (%)	ECF Tris (%)	Non-ECF Tris (%)	ECF Tris (%)	Non-ECF Tris (%)	ECF Tris (%)	Non- Tr (%
1	41	59	39	61	34	66	33	67	—	—
2	—	—	34	66	36	64	31	69	—	—
3	50	50	44	56	26	74	26	74	19	8
4	54	46	43	57	38	62	38	62	29	7
5	45	55	39	61	37	63	38	62	27	7
6	81	19	63	37	60	40	56	44	46	5
7	52	48	43	57	39	61	32	68	19	8
Mean	54	46	44	56	39	61	36	64	28	7

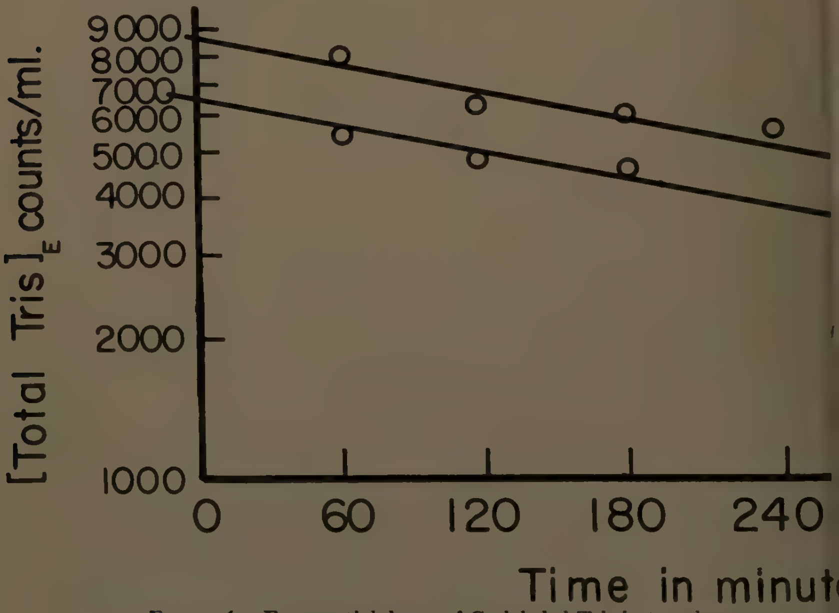

FIGURE 1. Exponential decay of C_{14}-labeled Tris in two dogs.

be defined as a process describing the distribution of weak acids or weak b across a membrane selectively "permeable" to the nonionized species. U these conditions, ratios of concentrations of the ionized form of a substanc the two sides of the membrane at equilibrium vary either directly or inver pending upon the charge carried by ionized species) as a function of the lrogen ion concentration ratios across the membrane. It can be seen from BLE 6 that at the end of 240 min., there is no correspondence between hydroion ratios and ionized $Tris^+$ ratios even assuming that all "nonextracellular" s is to be found within the "intracellular" compartment. These data are tative since there are a number of factors that have not been evaluated. example, possibly if more time were permitted for equilibrium, ultimately per ratios might be achieved. No data on protein binding of Tris are ilable. Appreciable plasma protein binding would modify the calculations he direction of better correspondence of ratios since (Tris)e would be smaller. n additional point of some importance is the fact that the pK_b of Tris in logical.fluids is not definitively established. As pK_b approaches plasma , the fraction of total Tris which exists as ionized $Tris^+$ diminishes. For mple, if pK_b is 1.0 U. greater than plasma pH, 90 per cent of total Tris sts as $Tris^+$; if pK_b is equal to plasma pH, 50 per cent of total Tris exists as

TABLE 6

ARENT FAILURE OF TRIS TO OBEY NON-IONIC DIFFUSION AT THE END OF 240 MINUTES OF EQUILIBRIUM

	$[(H^+)i]/[(H^+)e]$	$[(Tris)i]/[(Tris)e]$
1	2.89	1.50
2	2.76	0.65
3	1.70	1.08
4	1.86	1.04
5	3.55	0.77
6	4.90	0.41
7	3.02	0.83
Mean	2.96	0.90

s^+. Since intracellular pH is considerably lower than plasma pH, the acellular Tris is undoubtedly almost entirely present as $Tris^+$. These siderations indicate that if the pK^b of Tris is about 8.0 U., an appreciable nward correction of total plasma ionized $Tris^+$ concentrations should be de. This would tend to bring the observed ratios into accord with predicted os.

Summary

method has been briefly outlined that permits the determination of mean le body intracellular pH in the intact animal.

ntracellular pH averages 7.08 pH U. in the anesthetized dog under control ditions. The concentration of H^+ ion in intracellular fluid is more than ce its concentration in extracellular fluid.

ris appears to readily penetrate cell membranes and an important fraction otal buffering of this substance takes place inside cells.

oss of Tris from extracellular fluid takes place exponentially with time.

Although on the basis of present incomplete data, Tris does not seem penetrate cells by nonionic diffusion, further work is needed in this field.

A decisive area of acid-base metabolism concerns acid-base relations ins cells. Important advances in understanding the regulation of acid-base bala will undoubtedly stem from the refinement and application of techniq designed to study hydrogen ion activity changes in this area.

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CARBON DIOXIDE AND INTRACELLULAR HOMEOSTASIS

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I have been interested in electrolytes for a long time; my first paper on the bject was published from the laboratory of W. J. V. Osterhout in 1916, who ould be remembered as the man who first proposed a carrier mechanism for e active transport of potassium. Later, during World War I, it was my ivilege to work for a time in L. J. Henderson's laboratory, to which I had en assigned as a United States Army sergeant. It was easy to recognize the eatness of the man although at the time I knew too little about electrolytes d blood to have any real concept of the magnitude of his contributions to ence. This appreciation came after I had the opportunity of studying his ok on blood and learned that this was one of the most beautifully complete apters in all of physiology. At that time a few sophisticated persons could lk about pH, and more knew something about hydrogen ion concentrations, ich were still measured by a few experts with a potentiometer and a hydrogen ctrode. Electrolytes were of interest to a few specialists only and were of concern to clinicians in general. It was Henderson who first clearly recog- zed the remarkable properties of carbonic acid; the Henderson-Hasselbalch uation (by which we calculate pH) still bears his name. He wrote a book lled *The Fitness of the Environment* that included a discussion of the fitness of $)_2$ for biological purposes. Carbonic acid is a weak acid and is easily dis- aced from base by the addition of a stronger acid, thus providing a large ction of the buffering of the body. With this auspicious initiation into the bject of electrolytes I could hardly fail to maintain an interest in this field roughout my active career. This paper is something of a review of my long ation to the subject of tissue buffering.

One of the best measures of the buffering capacity of blood or tissues is the $)_2$ dissociation curve, which might be regarded as the result of titrating the ution or tissues with carbonic acid. As the CO_2 tension is increased, base is ken away from the other buffers and becomes available for combining, with arbonate. Carbon dioxide is always available to combine with any free se, and the bicarbonate content of a solution or a tissue may be regarded as neasure of the excess of fixed or nonvolatile cations over anions. The CO_2 sociation curve of blood is well known, but that of tissues is less familiar; erefore, I may well begin a review of tissue buffering at this point.

In so far as I know, the first CO_2 dissociation curves of tissues were those I scribed in 1928*a*, which I had investigated with the aid of a somewhat anti- ated method (FIGURE 1). The tissue to be analyzed was quickly introduced o the apparatus and subjected to grinding under acid. The CO_2 released s carried over to $Ba(OH)_2$, where it was absorbed and analyzed by the ctrical conductivity of the medium.

These CO_2 dissociation curves show that the curve for blood is much higher an that for muscle or nerve. Similar results were obtained by Stella (1929). e curve for heart muscle was even lower than the curve for skeletal muscle

(Brody, 1930); this seems to be true at least in the frog. Likewise wint tissues of frogs appear to bind less CO_2 than the same tissues in summer; a C dissociation curve for dog lung is also available (DuBois *et al*,. 1952).

The CO_2 dissociation curve for bone is, of course, much higher than any the others (Freeman and Fenn, 1953). This curve was based on experimen on rats. The bones of these animals were found to contain slightly less C after 2 weeks in a low oxygen environment and slightly more than the norm amount of CO_2 after 2 weeks in 10 per cent CO_2 mixture. In shorter perio of time no significant changes were observed. The percentage changes a

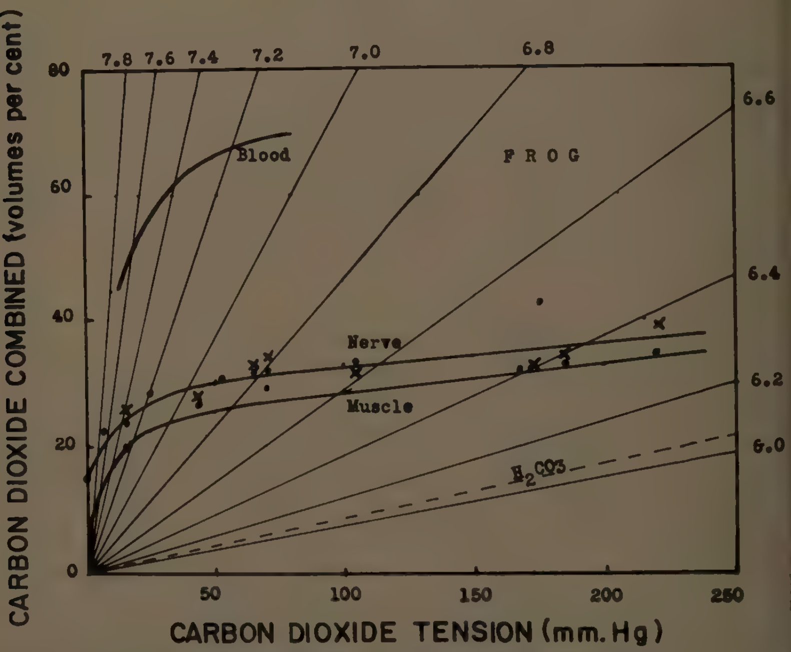


FIGURE 1. Carbon dioxide dissociation curves of frog nerve, muscle, and blood. T experimental points apply to the nerve curve. Reproduced by permission from *Medic* (1928*a*).

of course, small and the result needs confirmation. The curve is also differe from the other curves in that the bound CO_2 must be in the form of carbona instead of bicarbonate. Nevertheless there might well be some gain or l by exchange with the blood, and this has been invoked as an important sou of body buffer.

My original purpose in preparing these CO_2 dissociation curves was to obt evidence for the *p*H inside the muscles by use of the Henderson-Hasselba equation. This was, I think, the first application of this method to tissu Later (Fenn and Maurer, 1935) I made careful allowance for what I called t chloride space and corrected the *p*K value for the ionic strength of the c contents and the temperature, and I arrived at 6.9 as the *p*H inside a mus ll. This figure was substantiated later by Wallace and Hastings (1942). he result has been seriously questioned by Conway, who claims that much of e combined CO_2 is not bicarbonate because it is not precipitated by $Ba(OH)_2$. his result has not yet been confirmed by others. I tried to check it myself ice by determinations of the *p*K of a muscle-mash, but I was never quite tisfied that the results were good enough to publish. Anyhow the results did ot indicate anything wrong with the *p*K I had used and my figure of 6.9 for e *p*H has been confirmed by other methods based on the distribution of nmonia and, more recently, by methods based on the distribution of 5,5-methyl-2,4-oxazolidinedione (DMO) in the laboratory of Waddell and Butler 959).

Ever since the function of buffers was known, I suppose it has been realized at red cells and tissue cells might supply some of the buffering capacity. his was first shown by Van Slyke, (quoted by Peters and Van Slyke, p. 895) ho found that approximately 6 times as much acid was required for a given ange in the *p*H of the blood when the blood was acidified *in vivo* in contact ith the tissues. This is also shown by "titrating" the whole body with CO_2. hen an animal is made to breathe a high CO_2 mixture, a certain amount of tra CO_2 is absorbed, and the fraction of this that is found in the blood has en found to vary from 12.6 per cent (Shaw, 1926) to 33 per cent (Farhi and ahn, 1955). All the rest must have been somewhere in the tissues. F. H. eeman (1943) in my laboratory carried out similar experiments on rats. She ade no measurement of the gas absorbed by analyses of the expired air but ssolved the whole rat or samples of different tissues in acid and measured the O_2 that escaped. Furthermore, the equilibration period for 10 per cent CO_2 halation lasted about 2 weeks. Under these conditions only 5.9 per cent of e extra CO_2 was in the blood, 32 per cent was in the bones, and the remainder as found in the various other tissues of the body. By either method a large action of the extra CO_2 retained was found in the tissues. A curious fact as the finding of Irving *et al.* (1930) that in experiments with high inspired O_2 where the total CO_2 absorbed was estimated from the expired air, only out 30 per cent at most could be recovered by analyzing blood and soft ssues. The remainder might have been in bone, but the investigators could t be sure. If this is true it is a problem for the future.

We come now to the mechanism of this neutralization by tissues. Red cells ere originally supposed to be impermeable to cations; this left only movements chlorides or other anions for acid-base adjustment. When acid was added plasma the well buffered cells shared the load by accepting chloride from the asma.

This same general principle was applied to tissue cells at first until it was own that they seemed to be relatively impermeable to chloride but readily rmeable to potassium. It was natural therefore to suppose that most of e tissue buffering would be due to movements of potassium. Indeed I had trouble in showing (Fenn and Cobb, 1934) that with a preparation of frog uscle in Ringer's solution, acid would bring the potassium out while alkali ould move it in.

It is hard for me to explain why I did not examine the movements of sodium at the same time. Perhaps it was because I had it firmly in mind that muscl were impermeable to sodium, or perhaps it was because in those days analys had to be made by a laborious chemical technique, and I did not have enoug technical help. Later, however, we found, using radioactive sodium, that th sodium readily exchanged with all the muscle sodium, so there seemed reason why it could not participate in acid-base adjustments, as it probab does.

Not until many years later (Fenn *et al.*, 1958) did I get around to repeatin this experiment with sodium analyses in the PFP era (post flame photometer

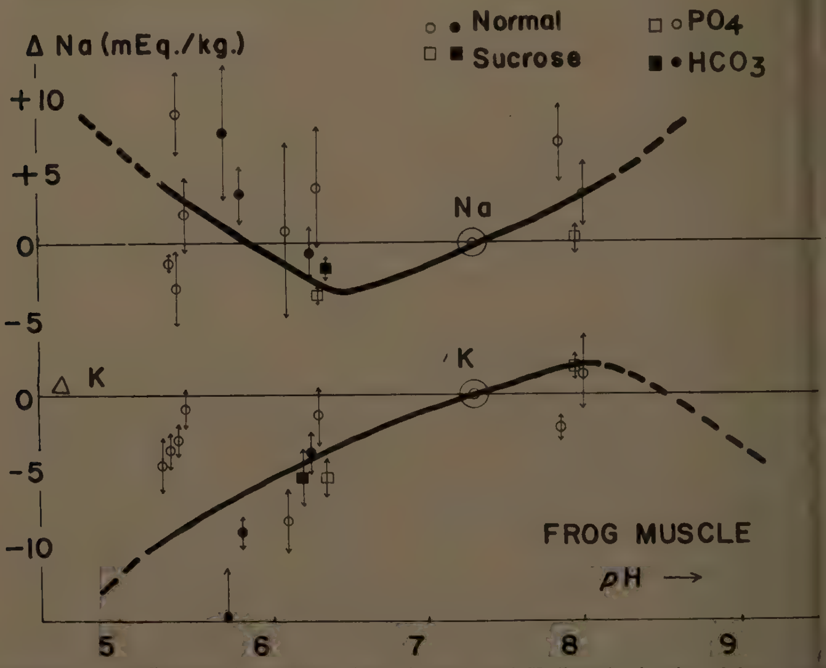


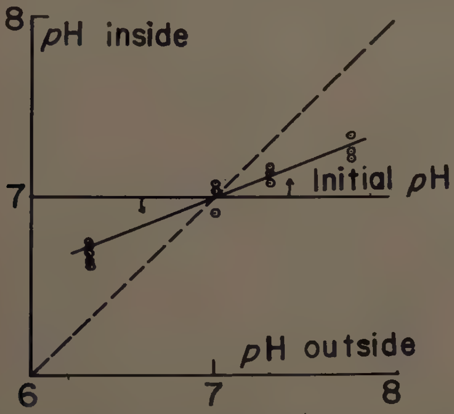
Figure 2. Differences in content of Na (*upper*) and K (*lower*) of pairs of frog musc immersed in Ringers solution, one member of each pair at pH 7.3 (circles), and the other a different pH as indicated on the abscissae.

The results for sodium were less convincing than for potassium, but there seem to be some movement of Na out of the muscle into acid solutions in m of the experiments (figure 2).

Such a movement of Na from tissues into acidified plasma has been nic confirmed by Swan and Pitts (1955) in dogs, by Tobin (1956) in cats, and Elkinton *et al.* (1955) in humans. The results reported in the first two of th papers showed that the percentage of injected acid neutralized by moveme of sodium (29 to 35 per cent) was much greater than that neutralized by mo ments of potassium (5 to 10 per cent). In analyzing the muscles themselv however, Tobin (1956) found actually more loss of potassium than of sodi and the loss of sodium by the muscles seemed equal to the gain by the ext cellular fluid. Only the fate of the K lost by the muscles seemed to be

t. Some have thought that much of the Na appearing in the ECF might from the bone; Bergstrom and Wallace (1954) have reported losses of m from bone under such condition, but Forbes *et al.* (1959) at Rochester, ., seemed unable to confirm this. The question is still open, therefore, ugh it is evident that muscle sodium does participate to some extent.

his muscle sodium, however, may be in the more superficial parts of the le and indeed there are some investigators, for example, Simon *et al.* (1957), believe that the cell water may be divided into potassium water and m water, the sodium being for some reason unable to penetrate into the r in close association with the myosin, which is conceived of as having a of crystal structure into which potassium can fit while sodium cannot. is a useful hypothesis, but I am still inclined to prefer the sodium pump (originated by R. B. Dean in my laboratory) with all its imperfections.



GURE 3. Values calculated for the *p*H of the inside of a muscle after 5 hours immersion utions of different *p*H values as indicated on the abscissae. The *p*H inside the muscle alculated from the CO_2 content by the Henderson-Hasselbalch equation. Reproduced rmission of the *Journal of General Physiology* (Fenn and Cobb, 1934).

rding to this hypothesis the membrane appears to be impermeable to m because that ion is pumped out as fast as it gets in by some sort of a er mechanism that can discriminate between K and Na.

nally there is the possibility of some movement of chloride into the cell acid is applied outside although at best there is very little chloride inside, of it being in what we called the chloride space outside. Considering paucity of chloride it is rather remarkable that there should not be an s of bicarbonate in muscle and an alkaline rather than an acid intracellular r. There are, however, plenty of protein, phosphate, and other anions mbine with all the base present so that there is relatively little left for bonate.

direct proof of the buffering power of muscles I mention an experiment 26 years ago (Fenn and Cobb, 1934). It is of interest because not many urements of intramuscular *p*H have been made even now, and this shows hanges that occur when the *p*H outside is made to vary (FIGURE 3). In ourse of an immersion of 5 hours the muscles in alkaline solution gained bicarbonate and pH increased, whereas those in acid solutions lost bicarbo and pH decreased. These effects are known to reflect to some degree co sponding movements of potassium although movements of other cations of anions are certainly involved.

As I have indicated, in isolated frog skeletal muscle most of the acid- adjustments seem to occur by means of potassium movements. This, howe is not necessarily true within the body. One of the differences is that in body the muscles are in equilibrium with blood or with the true plasma of blood. In order to see what effect this might have, I once varied my exp ment by immersing frog muscles in whole frog blood and then tested the e of increasing the carbon dioxide tension from 1 to 20 per cent of an atmosp (Fenn and Cobb, 1935). The results were surprising since the potassium tent was slightly but significantly increased by the high CO_2 tension when muscle was immersed in blood,* whereas it was always markedly decre when the muscle was in Ringer's solution (TABLE 1). After this perio

TABLE 1

PAIRS OF FROG MUSCLES AT 1 AND 20 PER CENT CO_2 AFTER 5 HOURS IMMERSION AT 2
IN FROG BLOOD OR RINGER'S SOLUTION
(mEq./kg.)

Immersion fluid	1 per cent CO_2		20 per cent CO_2		Difference (per cent)	
	K (mEq./kg.)	HCO_3 (mEq./kg.)	K (mEq./kg.)	HCO_3 (mEq./kg.)	K (mEq./kg.)	HC (mEq.
Blood	78.0	8.4	83.0	12.4	+5.0	+4
Ringer's solution	73.1	4.1	69.6	2.8	−3.5	−1

immersion the muscles were put into respirometers in a 5 per cent CO per cent O_2 mixture, and their CO_2 content was measured by acidification a equilibration was complete. The muscles that had been in the high mixture previously always contained an increased amount of CO_2 that c be accounted for partly by the increased K content. Similar experiments later repeated with Gerschman (Fenn and Gerschman, 1950) using frog ner and the same result was obtained, although with rather less statistical certai

The interpretation of these experiments seems to be that the potassium m toward the phase where the CO_2 has produced the greatest increase in th ion concentration, that is, it follows the Donnan equation $H_i/H_o = K_i/$ The increases in the concentrations of H^+ in true plasma, muscle, and Rin solution when CO_2 is increased were calculated to be 9.6×10^{-8}, 29×10^{-8}, 73×10^{-8} respectively. In this blood-muscle system, therefore, incre tension drives chloride into the red cells and drives potassium into the mu This is because red cells with their plasma form a better buffered system muscle cells with their extracellular fluid. The red cells prevent H_o in sma from increasing with high CO_2 tension as much as H_i inside the muscles, refore K_i must increase relative to K_o.

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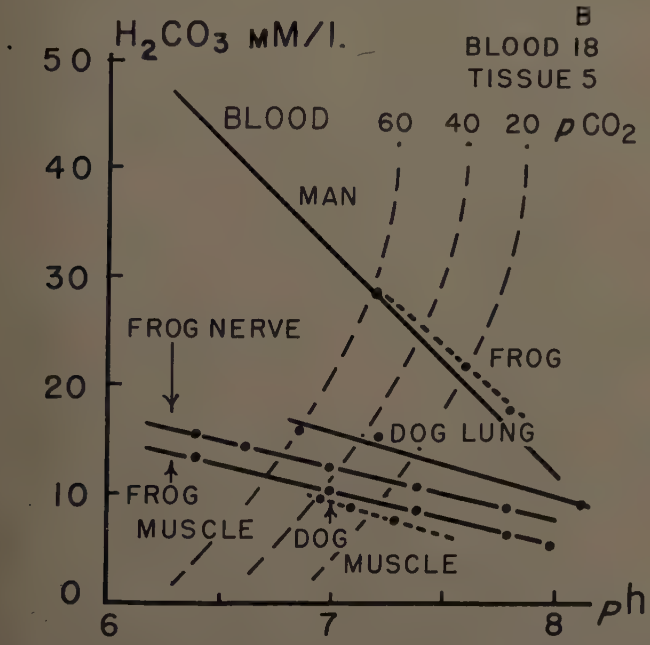
* The high CO_2 in blood did not actually drive K into the muscles, but it greatly dimin the loss of K that always occurs in isolated muscles even when immersed in blood.

his is the situation for equilibration with a high CO_2 tension. When fixed d is added to plasma and not to tissues K would be expected to come out and e versa. Likewise if acid were formed inside muscle cells it would either use out into the plasma or it would tend to bring potassium into the muscle m the plasma.

s I have indicated, the buffering capacity or dB/d *p*H is greater in muscle n in Ringer's solution, and it is greater in blood than in muscle. This is



IGURE 4. Buffer lines for blood and tissues. Dotted lines show the value of CO_2 ten-. Increasing CO_2 tension to the left decreases the *p*H and increases the bicarbonate ent.

l illustrated by plotting the buffer curves on a Davenport diagram with rbonate content plotted against *p*H (FIGURE 4). In addition to data of my n I have included the curve for dog lung obtained from data by DuBois l. (1952) and data for dog tissues in general calculated, with certain assump-s, from data of Farhi and Rahn (1955). The value of dB/d *p*H is given by slope of these curves, which is obviously much greater for blood than for scles. Since the total bicarbonate content of tissues is less than that of od it is obvious that they must be more acid at equal CO_2 tensions as shown the curve of the pCO_2 lines. It can be seen that the pCO_2 = 40 line inter-s the blood line at *p*H 7.4 and the muscle line at *p*H 7.0. Likewise it may seen that if the CO_2 tension is increased from 40 to 60 mm. (along the blood or tissue buffer line) the amount of a buffer such as THAM that woul required to bring the pH back to the normal value (by movement up alon $pCO_2 = 60$ line) would be far greater for blood than for the tissues. If, t fore, THAM is evenly distributed between blood and tissues and if the pH remains normal, then the tissues must be overneutralized and potas would tend to move out into the plasma.

This same fact is shown by FIGURE 5, where essentially the same data plotted on a classic CO_2 dissociation curve modified from Fenn and (1935). At a pCO_2 of about 4 per cent the pH of the muscle is 7.0 and th the true plasma is 7.4. When the pCO_2 is increased to about 6 per cent

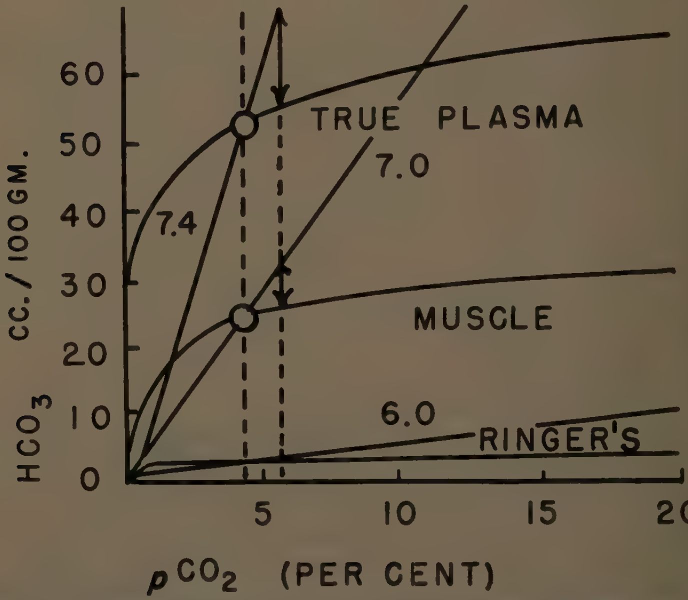


FIGURE 5. CO_2 dissociation curves of true plasma and muscle modified from Fen Cobb (1935). The solid double arrows show the amounts of THAM required to bri pH back to normal after the pCO_2 is increased from one dotted line to the other.

is, from one dotted line to the other) the CO_2 content of both muscle and pl increases along its appropriate curve. To restore the original pH value CO_2-combining capacity must be increased by THAM or otherwise alon vertical arrows. The length of this arrow is greater for true plasma tha muscle.

This phenomenon of overneutralization in the tissues will affect the am of THAM required to maintain constant blood pH when a given amou CO_2 is retained.

Considering only the blood at pH 7.4 where THAM is 80 per cent io the amount of THAM required per mole of CO_2 absorbed by the blood be 1.2; considering only the cells at pH 7.0, it would be 1.1 since ther THAM is 90 per cent ionized. If, however, the un-ionized THAM is eq tributed in all the body water the cells would be overneutralized and an ormally large fraction of the CO_2 absorbed by the whole body would be in cells. As a result the potassium might move out of the cells to restore the rmal Donnan ratio. Still a little more than 1 mole of THAM would be uired per mole of CO_2. If now THAM did not penetrate cells at all, then ch unneutralized CO_2 would be in both red cells and muscle cells although plasma *p*H was kept at 7.4. Under such conditions the ratio of THAM to $_2$ absorbed would be 1.1:3 = 0.37 assuming that two thirds of the absorbed $_2$ was in the tissues and unneutralized, and assuming no migration of K or or other electrolytes between tissues and plasma. The fact that this ratio found experimentally to be a little greater than 1, according to Nahas, ports his conclusion, obtained by direct analysis, that the volume of distribu- n of THAM is great enough (50 to 65 per cent of body water) to neutralize tissue CO_2 as well as the blood CO_2. THAM does not need to penetrate all the body water and overneutralize the cells in order to attain this ratio. This demonstrated ability of THAM to diffuse into cells to just the right

TABLE 2
BUFFER CAPACITY PER KILOGRAM BODY WEIGHT*

	B†	V‡	BV
Blood	18	0.10	1.8
Tissues	5	0.65	3.25

* BV.

† $B = \dfrac{\Delta \text{mEq./kg. of tissue}}{\Delta\, p\text{H}}$

‡ $V \doteq \dfrac{\text{kg. of tissue}}{\text{kg. body weight}}$

ent must be one important reason for its efficacy as a buffer. If the same ect were tried with NaOH, which does not easily penetrate cells, then the sue CO_2 would be unneutralized unless an equal amount of K moved in or moved out to achieve the same result. In this process less NaOH than HAM would be required per mole of total CO_2 absorbed.

If the buffer capacity per kilogram is multiplied by the total weight of the sue in the body, the total buffer capacity of that tissue in the body is obtained indicated in TABLE 2. This shows that muscle has approximately one third buffer capacity of blood per kilogram, but as there is 6 times as much of h tissues as blood the total buffer capacity of the tissues is about twice that blood. This agrees with the results of Farhi and Rahn (1955), who reported o thirds of the CO_2 retained in hypercapnia as being in the tissues.

Comparisons of *p*H changes or buffer capacity ($dB/d\,p$H) between muscles d blood are not entirely satisfactory as a basis for predicting movements of tassium which, according to theory, should depend upon the ratio of the drogen ion concentration inside to outside or $H_i/H_o = r$, the Donnan ratio. theory it is also true that $r = K_i/K_o = (HCO_3)_o/(HCO_3)_i$. Assuming validity of this last equation the changes in r that would result from an

increase in $p\mathrm{CO_2}$ would depend not on absolute increments in $\mathrm{HCO_3}$ in c
and blood but on percentage increments. Since there is nearly twice as m
$\mathrm{HCO_3}$ in blood as in muscles, the increment in $\mathrm{HCO_3}$ must be more than tw
as great in blood as in muscle to increase the value of r. Thus after inha
$\mathrm{CO_2}$ the new value of r or r' would be $r' = r\,(100 + S_o)/(100 + S_i)$ wher
is the percentage increment in $\mathrm{HCO_3}$ per millimeter Hg increase in pC
Taking S_o as applying to the true plasma curve it includes the effect of the
blood cells in buffering the plasma. If this is greater than the increment
S_i for the same increment in $p\mathrm{CO_2}$ then r' would be greater than r and K_i wo
tend to increase.

In accordance with this theory I have estimated the values of S for bl
and tissues as shown in TABLE 3 and find again that the value for blood (1
is greater than that for muscle (0.7). For an increase of 10 mm. Hg in pC
the $\mathrm{HCO_3}$ in the plasma would be 110 per cent of its former value whereas
muscle would be 107 per cent. To restore r to its original value, therefore,
amount of K equivalent to about 3 per cent of the plasma $\mathrm{HCO_3}$ or about
mEq./l. should move into the cells. Nevertheless an easily detectable decre
in plasma K with an increase in pH or vice versa has been well documen

TABLE 3

PERCENTAGE INCREASE IN $\mathrm{HCO_3}$ PER MM. HG INCREASE IN $p\mathrm{CO_2}$ AT $p\mathrm{CO_2}$ = 40

Dog true plasma	1.0
Dog muscle	0.7
Frog true plasma	0.5
Frog muscle	0.23

(for example, Simmons and Avedon, 1959); this perhaps throws doubt upon
validity of the whole Donnan equilibrium theory of body electrolytes. T
theory therefore must be held with some reservations, although it appears
be very useful as a guiding principle; exceptions may be due to unknown c
flicting processes. The theory is a little hard to apply because in mus
$\mathrm{K}_i/\mathrm{K}_o$ appears to be greater than measured values of $\mathrm{H}_i/\mathrm{H}_o$. Likewise
does not seem applicable to the movements of Na, which seem to depend u
the effects of pH on the sodium pump. In frog skin the sodium pump
inhibited when it has to pump into a more acid medium, but in muscle one m
suppose the reverse to be true if the movements of Na are to be explained
this basis.

According to theory, then, an increase in $p\mathrm{CO_2}$ should cause K to move fr
the plasma into the cells. In attempting to verify this in cats, Fenn
Asano (1956) found that the situation was obscured by a liberation of adre
ine, which mobilized K from the liver along with glucose. With concentrati
of $\mathrm{CO_2}$ below the threshold for this reflex, however, we did find a fall in plas
K, and we regularly found a sudden increase in plasma K when the p
returned to normal. This seemed to support the theory, but it does not
us that this is the only mechanism that controls acid-base balance between c
and tissues. Both renal and metabolic influences must be considered. M
investigators have found plasma K increased after respiratory acidosis; thi

t the opposite of the effect predicted and the opposite of the effect found in lated frog muscle.

Tobin (1956) found that the pH of the muscle water did not change even en the pH of the plasma was lowered by HCl injection, and he looked for tabolic changes that would serve to regulate the pH of the inside of the cell ependently of the Donnan ratio and the pH of the plasma. Lipmann and yerhof (1930) found that in muscles a high CO_2 tension caused a metabolic akdown of phosphocreatine which would tend to neutralize the acidity caused carbonic acid. Tobin (1960) could not find that this occurred in his prepara- n, but he did find decreases in lactic acid in alkalosis. Another possibility metabolic buffering is the increase in concentration of lysine as a cation orted by Eckel *et al.* (1958) in the cellular acidosis and alkalemia resulting m potassium deficiency. Likewise there might be changes in the concentra- n of ammonia, carnosine, or anserine, or changes in intermediates of the ric acid cycle that could have some acid-base significance. Long-term effects tainly involve the kidney more than the cells, because the kidney will ays be the final influence on the composition of the blood.

conclude this paper by pointing out that we have only one well-worked- theory of the mechanism of tissue buffering. This is the Donnan equilib- m theory, which works out better for isolated muscles than it does for the ch more complicated situation of muscles in the body. In the body, there many complicating factors that sometimes obscure the underlying opera- n of the physicochemical equilibrium between muscles and blood. The erior of cells is not a medium of uniform composition but may have to be arded as consisting of two or more separate phases. The pH of the cells y be maintained constant by metabolic reactions in spite of changes in them nanded by the Donnan ratio. All the equilibria within the body are dy- nic equilibria and not static, and the cells must be continuously adjusting many different demands. When we focus our attention solely upon the d-base changes, we become blind to the adjustments required for carbo- lrate supply or hormone influences, emotional states, hunger and thirst, ly temperature, and bodily activity in general. Therefore we must not ect too much of any single theory. The amine buffers seem to fit into the eral scheme of things rather well. THAM is a good buffer because its pK n the right range; it is relatively nontoxic; it penetrates cells fairly easily; l it acts as a diuretic and is rapidly excreted. There is nothing miraculous ut its actions, most of which can be predicted fairly well from its chemistry l the general theory of acid-base changes. We have, however, much to n about this subject; we are in real need of a better theory if there is one be had. We may well hope that this monograph on amine buffers will vide an exchange of ideas that will speed us on our way toward this goal.

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art IV. Physiological Effects of Amine Buffers

EFFECTS OF AMINE AND OTHER BUFFERS ON RENAL ACID EXCRETION*

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The effectiveness of an alkalinizing agent for *in vivo* use depends upon several ctors, including chemical properties and maximum possible rate of infusion termined by the toxicity and locus of distribution. The duration of effec- veness also depends on several factors, such as rate of diffusion into various dy compartments, metabolic rate, and rate of excretion. In addition, the gree and duration of the effect will also be partially determined by influence the rate at which the kidney excretes acid or base. The greater the induced crease in base excretion (or decrease in acid excretion) the shorter time the kalizing compound will be effective.

One compound recently found to be effective as an alkalinizing agent is tris- ydroxymethyl)aminomethane, commonly called Tris.[1] The purpose of this udy was to compare the effect of Tris on renal acid excretion with the effects l-arginine, another organic compound, and sodium bicarbonate, an inorganic mpound, to determine: (1) if differences exist between the effects of organic d inorganic compounds on acid excretion, and (2) if Tris has the same effect another organic compound with somewhat similar physicochemical prop- ties.

Methods

Thirty experiments were conducted on female dogs anesthetized with sodium ntobarbital (25 mg./kg., with additional doses as needed). Ventilation was aintained constant (with a Harvard respiration pump and small doses of ccinylcholine chloride for muscle relaxation) at a rate of 12 respirations per inute and a tidal volume adequate to maintain arterial *p*H at approximately 40.

When a steady state was reached, as indicated by constant arterial *p*H, two -min. control periods were run, followed by three 30-min. experimental riods during which either 0.27 *M* Tris, 0.25 *M* arginine, or 0.15 *M* sodium carbonate were infused at the rate of 0.3 ml./min./kg. (81, 75, and 45 μM/ n./kg. respectively.) Each of these rates of infusion had previously been termined to be adequate to raise blood *p*H approximately 0.20 units during e 90 min. of infusion. Urine was collected anaerobically at the end of each riod through an indwelling catheter using manual bladder compression, and aerobic blood samples were drawn from the femoral artery at the mid-point

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of each period. Each of the compounds was tested in 5 different experiment
This series of 15 experiments will be called the "constant pCO_2" series.

In a second series of 15 experiments the procedure was duplicated (5 T
infusions, 4 arginine, 6 bicarbonate) except that immediately on starting i
fusion of the alkalinizing solution, ventilation was decreased (the anim
breathing 100 per cent oxygen to prevent hypoxia) at a rate appropriate to kee
arterial pH constant as determined by measurement every 5 to 10 min. Ve
tilation was decreased further at 10- to 15-min. intervals by empirically d
termined increments, and arterial pH was thereby held within 0.03 pH unit
control values. This technique superimposed a respiratory acidosis on t
metabolic alkalosis of the infusion. These two acid-base disturbances we
opposite in direction but equal in degree in terms of their effect on blood p
This technique provided a method of determining the renal response to t
three infused compounds during a standardized respiratory acidosis, one ad
quate to maintain arterial pH constant during infusion of the alkalinizing sol
tion. This second series of experiments will be called the constant pH serie

Anaerobic blood and urine samples were analyzed for CO_2 (Van Slyke app
ratus) and for pH (37° C.; Beckman model GS pH meter; corrected to esopha
eal temperature by 0.015 pH unit/°C.[2]). Arterial pCO_2 and plasma and uri
bicarbonate concentrations were calculated with the Henderson-Hasselbal
equation.

Urine titratable acid concentration was determined by rapid titration
arterial pH within a few minutes of obtaining the sample, and urine ammon
concentration was determined by the steam-distillation method of Jenden a
Taylor.[3] The sum of the rates of titratable acid and ammonia excretio
(concentration x urine flow) was considered the rate of renal acid excretio
Creatinine clearances were determined by standard techniques using the san
blood and urine samples. The filtered load of bicarbonate was assumed to
the product of plasma bicarbonate concentration and creatinine clearanc
Reabsorbed bicarbonate was the difference between the filtered load and t
rate of excretion and was calculated as mEq./100 cc. of glomerular filtra
(creatinine clearance) per kilogram body weight.

Results and Discussion

Blood. The effects of the infusions on blood pH, pCO_2, and bicarbonate co
centration are shown in FIGURE 1. In order to eliminate interdog variabilit
mean changes from the last control period to each of the three experiment
periods are plotted for each type of infusion rather than absolute values. Ea
point represents the mean of all determinations for comparable periods (5 in
cases except arginine and bicarbonate constant pH experiments, where 4 and
values are averaged respectively). The standard error of the mean is sho
for the third infusion period only.

In the constant pCO_2 series, blood pH rose approximately 0.20 unit a
plasma bicarbonate concentration rose from 6 to 12 mEq./l. The pCO_2
mained constant within approximately 3 mm. Hg.

In the constant pH series, mean pH remained within 0.02 of control valu
while pCO_2 rose between 21 and 25 mm. Hg. and plasma bicarbonate conce
tration rose 10 to 15 mEq./l.

In the constant pCO_2 series, plasma bicarbonate concentration rose more llowing bicarbonate infusion (12.2 mEq./l.) than following infusion of either ris (8.7 mEq./l.) or arginine (6.3 mEq./l.). The filtered load of bicarbonate se considerably more following Tris infusion (33.7 mEq./min./kgm.), how-

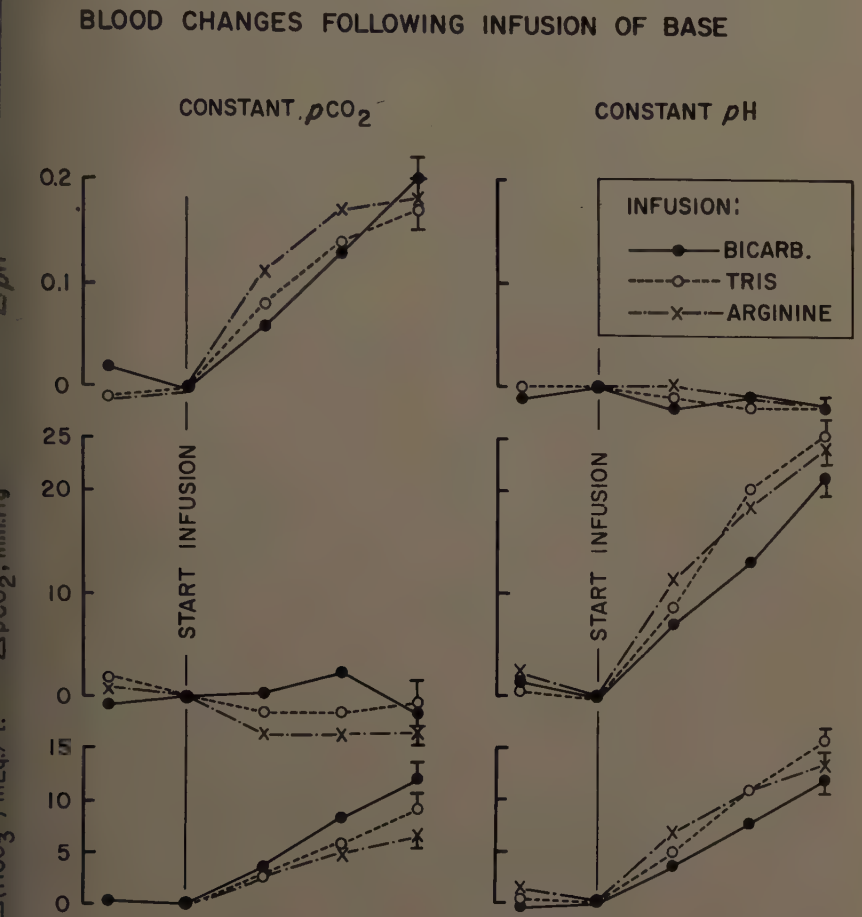


FIGURE 1. Changes in arterial blood pH, pCO_2, and bicarbonate concentration following usion of sodium bicarbonate, Tris, or l-arginine while either arterial pCO_2 or pH is held nstant. Each point represents the mean of 5 values for corresponding periods of infusion, ch approximately 30 min. (except 6 bicarbonate and 4 Tris experiments with constant pH). andard error of the mean is shown for the final infusion period.

er, than following infusion of either bicarbonate (9.0) or arginine (3.9), incipally because of differences of changes in filtration rate (TABLE 1.)

In the constant pH series, bicarbonate concentration rose significantly more lowing infusion of Tris (15.4 mEq./l.) and arginine (13.7 mEq./l.) than in the nstant pCO_2 experiments, as might be expected from the mass action effect an increase in pCO_2. However, plasma bicarbonate concentration did not e more following infusion of bicarbonate (11.7 mEq./l).

Urine. In the constant pCO$_2$ series, mean urine pH rose significantly mo following infusion of bicarbonate than either Tris or arginine (FIGURES 2 ar 5). On the other hand, the decrease in the rate of acid excretion (or increa in the rate of base excretion) was significantly less in the case of bicarbona

TABLE 1

CHANGE IN CREATININE CLEARANCE (GFR) FROM LAST CONTROL TO LAST INFUSION PERIOD (ml./min./kg.)

	Constant pCO$_2$		Constant pH	
	Control	Change	Control	Change
Bicarbonate	3.2	−0.54*	4.4	−0.23
Arginine	3.5	−0.68*	3.2	+0.06
Tris	2.9	+0.31	2.8	+0.45

* Statistically significant change.

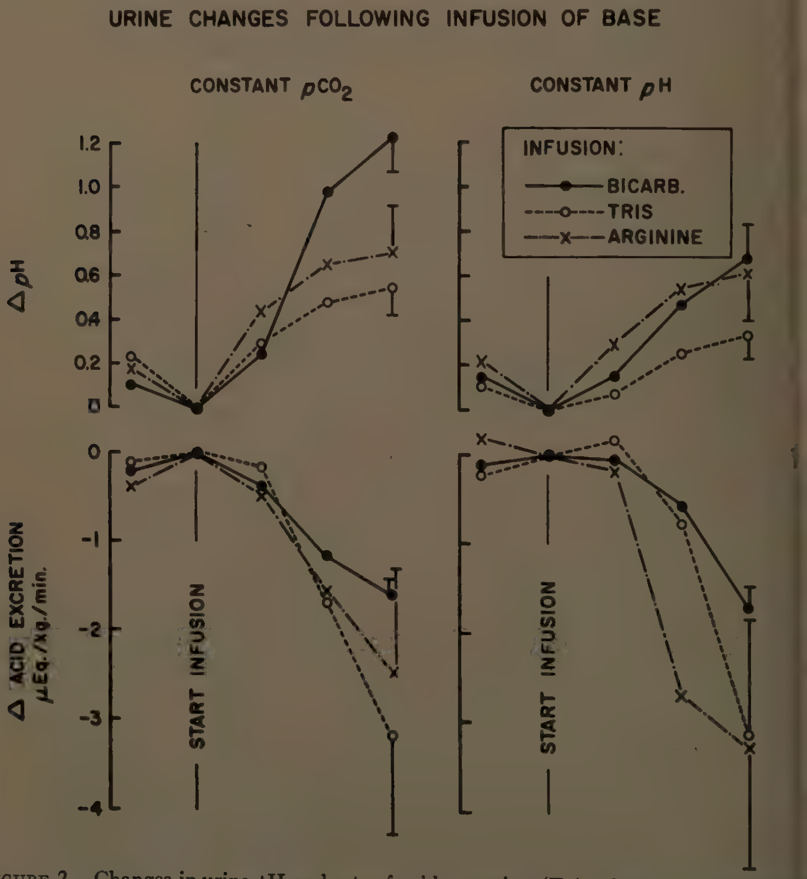


FIGURE 2. Changes in urine pH and rate of acid excretion (T.A. plus NH$_4^+$). See FIGU for further details.

53 μEq./min./kg.) than following either Tris (3.14) or arginine (2.44) in-
ion.

These data indicate that the two organic compounds behaved similarly and
ited a greater decrease in the rate of acid excretion (or increase in the rate
base excretion) than did bicarbonate. From the standpoint of this one
tor, they would therefore appear less effective agents for sustaining prolonged
alinization than bicarbonate. The maximum differences between the ef-
ts of bicarbonate and the organic compounds on the rate of acid excretion
re roughly 1 to 1½ μEq./kg./min., equivalent to 1½ to 2 mEq. per day per
, a moderately large acid load.

It is interesting that the bicarbonate infusion had more effect on urinary *p*H
t less effect on acid excretion than the other compounds. This can be ex-
ined only by a greater excreted load of buffer following Tris or arginine in-
ion, since *in vitro* addition of acid to urines collected during the third infusion
riod to return the titratable acid to levels of the last control period could
use a greater *p*H change with addition of less acid in the case of the bicar-
nate experiments only if there were less buffers in these urines.[4]

In the constant *p*H series, during a standardized respiratory acidosis, urine
again rose in 14 of 15 experiments in spite of the fact that arterial *p*H re-
ined constant, consistent with our previous observation that acute meta-
lic acid-base disturbances have more influence on renal acid excretion than do
nparable respiratory disturbances.[5] Again, the greatest increase in urine
occurred following infusion of bicarbonate (FIGURES 2 and 7) although the
rease was not statistically significantly greater than that for arginine, and
in the decrease in acid excretion was significantly less following bicarbonate
usion (1.71 μEq./min./kg.) than following infusion of either Tris (3.09) or
inine (3.21), which once more elicited similar responses. During respiratory
dosis, bicarbonate again appears to be more efficacious in sustaining alkalini-
ion from the standpoint of its effect on acid excretion than either of the
anic compounds.

These differences are also demonstrated in FIGURES 3 and 4. In FIGURE 3,
a for the $p\mathrm{CO}_2$ series are plotted. The change in arterial *p*H (stimulus) on
abscissa is plotted against the change in acid excretion (response) on the
inate for each infusion period for each of the 15 experiments. Regression
es fitted to the points for each type of infusion are shown. It is clear that
change in acid excretion for a given change in blood *p*H is similar for both
anic compounds and is less for bicarbonate than for either of these or for
two organic compounds together. These conclusions are borne out by
lysis of the data by statistical methods. It is also apparent that the rela-
ns may not be truly linear, although straight lines have been fitted because
amount of available data does not justify evaluation by curvilinear regres-
ns. The renal response appears to be greater per unit *p*H change with larger
ds of the alkalinizing compounds or in the last of the three experimental
iods.

Similar data for the constant *p*H series are shown in FIGURE 4. The change
cid excretion is significantly greater for arginine than for bicarbonate but is
significantly greater for Tris than bicarbonate because of one experiment
which the urinary changes were opposite to those of the other 14 experiments.

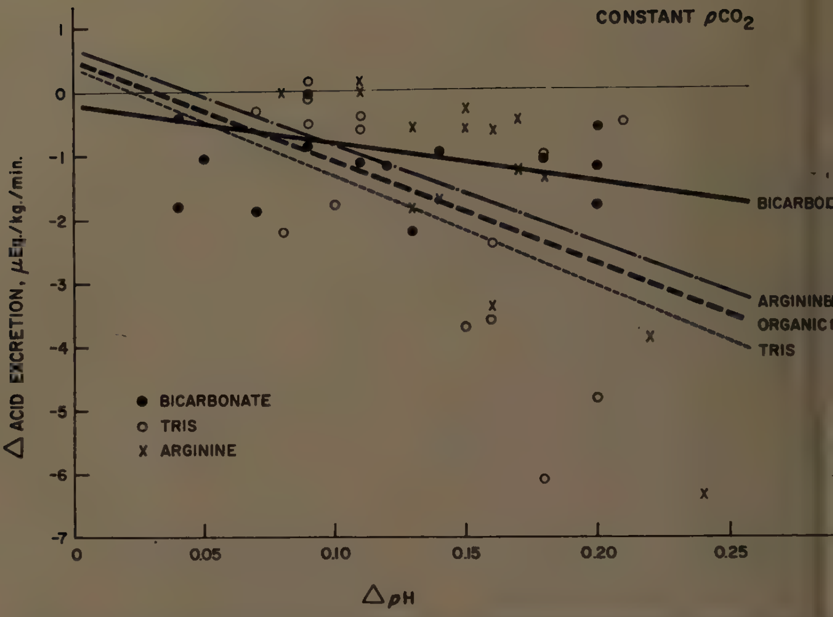


FIGURE 3. Change in renal acid excretion as a function of change in arterial pH fo
dividual clearance periods of constant pCO$_2$ experiments for three types of infusion.
shown are fitted by method of least squares. Line labeled "Organic" is fitted to all p
for Tris and arginine experiments.

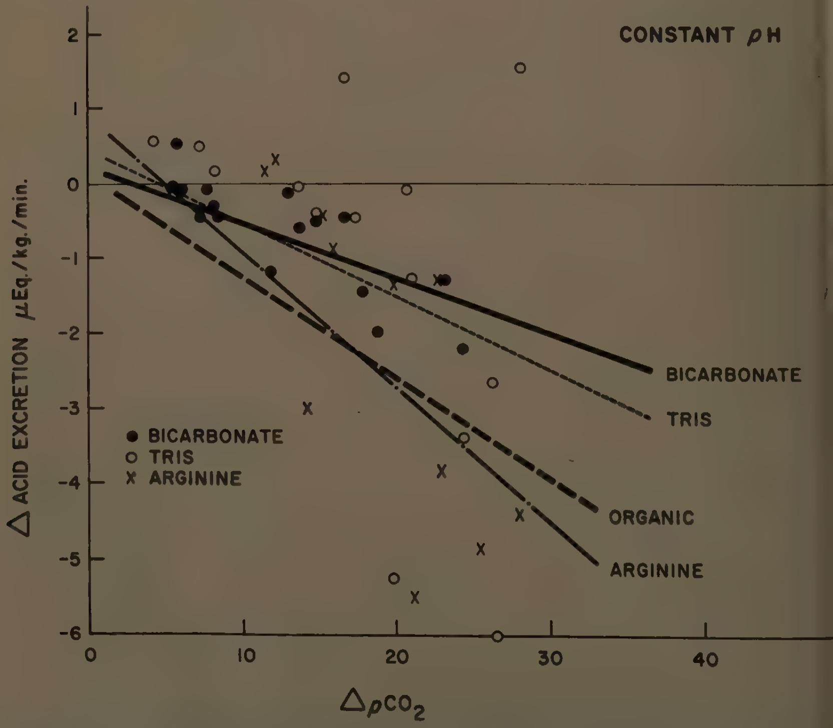
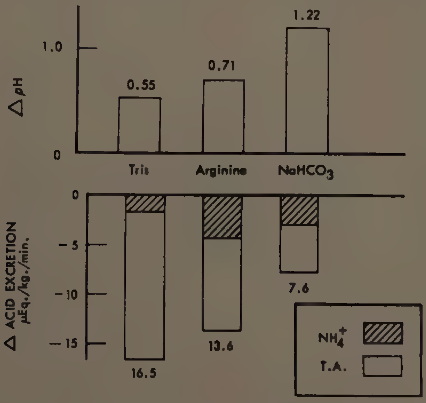


FIGURE 4. Change in renal acid excretion as a function of change in arterial pCO$_2$ ir
stant pH experiments (see FIGURE 3 for further details).

his one aberrant experiment were eliminated, the same results would be
nonstrated as in the experiments without respiratory acidosis, namely, that
ırbonate causes significantly less change in acid excretion for a given change
ırterial *p*H than do either of the two organic compounds, which do not differ
nificantly from each other. If Tris and arginine data are combined in this
es of experiments, a significant difference is demonstrable between the effect
bicarbonate and the effect of both organic compounds on acid excretion.
ince the response appeared to be greatest for a given blood *p*H change in
h series of experiments in the last of the three infusion periods, the same
a are presented for the third infusion period only in FIGURES 5 and 6. In
er to correct the data for the fact that the same stimulus (change in *p*H or
O_2) did not exist in each case, the change in rate of acid excretion is expressed
unit change in blood *p*H in the constant pCO_2 series or per unit change in
od pCO_2 in the constant *p*H series. In addition, FIGURES 5 and 6 demon-



IGURE 5. Changes in urine *p*H and rate of acid excretion for final infusion period of
stant pCO_2 experiments. Change in rate of acid excretion is expressed per unit change
rterial blood *p*H.

te the partitioning of the changes in acid excretion between ammonia and
atable acid. It is apparent that the greater part of the changes were due to
nges in titratable acid, presumably because of the relatively large amount
buffer entering the urine. As a matter of fact, calculation of changes in
atable acid accounted for by phosphate indicates that at least 85 per cent of
change must be associated with nonphosphate urine buffers, presumably
buffer being infused and excreted.* It is also of interest that the decrease

The change in titratable acid accounted for by phosphate was calculated from the equa-
:

$$H_2PO_4^-) = U\ (\text{total PO}_4)\left(\frac{1}{\text{antilog}\ pH_u - pK) + 1} - \frac{1}{\text{antilog}\ (pH_b - pK) + 1}\right)$$

re $(H_2PO_4^-)$ = excretion rate of $H_2PO_4^-$(μEq./min./kg.) in either the last control or last
sion period; U = urine flow; total PO_4 = excretion rate of phosphate (μM/min./kg.);
= urine pH; pH_b = arterial blood pH; and pK (6.8) is for the reaction $H_2PO_4 \leftrightarrow H^+ +$
$)_4^=$.
he change in titratable acid due to phosphate is then $(H_2PO_4^-)$ for the last infusion pe-
minus $(H_2PO_4^-)$ for the last control period.

in ammonia excretion following arginine infusion was greater than for either the other two compounds, consistent with the observation that arginine is a precursor of urinary ammonia in the dog.[6] FIGURE 5 again demonstrates t fact that bicarbonate infusion resulted in a greater change in urinary pH b less change in acid excretion than either of the other compounds, which w quite similar. FIGURE 6 demonstrates similar results in the constant series. Again, the change in pH is greater for bicarbonate than for the othe but the effect on acid excretion is less. The increase in urinary pH is also l for each of these three compounds in this series than in the constant pC series, but only moderately so. Similarly, there is slightly less effect on a excretion following Tris and arginine infusions, but no significantly differe effect following bicarbonate infusion as compared with the constant pC series. These data therefore indicate that bicarbonate infusion has the le effect on acid excretion during a respiratory acidosis as well as during the n mal respiratory state. Furthermore, the fact that there is relatively little c

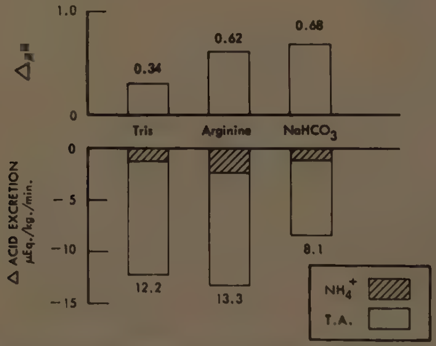


FIGURE 6. Changes in urine pH and rate of acid excretion for final infusion period of c stant pH experiments (see FIGURE 5 for further details).

ference between the effects in the constant pH and pCO_2 series again bears the previous observation that the respiratory acidosis has very little influe on acid excretion compared with the effect of the metabolic alkalosis.[5]

Changes in the rate of acid excretion have been shown to be closely rela to the filtered load of bicarbonate following HCl or $NaHCO_3$ infusion.[5] In t series of experiments, the relationship was poor, presumably because acid cretion should be expected to be related to the filtered load of all buffers, wh differed markedly from the filtered load of bicarbonate with Tris or argin infusion.

Bicarbonate reabsorption. Changes in bicarbonate reabsorption, expres as mEq./100 cc. glomerular filtrate are presented in FIGURE 7. As might predicted from the effects on acid excretion, bicarbonate infusion resulted in increase in bicarbonate reabsorption which was equal to or greater than effects of the organic compounds in both series. This greater conservation bicarbonate implies a more prolonged alkalinizing effect of bicarbonate fusion.

It is of interest that for each type of infusion bicarbonate reabsorption eased more in the constant pH series than in the constant pCO_2 series, pre-mably because of the fact that the increased pCO_2 of the former has been own to enhance bicarbonate reabsorption.[7-9]

It is interesting that bicarbonate reabsorption increased following bicarbon-e infusion in the constant pCO_2 series, since it has previously been reported at changes in the filtered load of bicarbonate due to bicarbonate infusion do ot affect bicarbonate reabsorption expressed in the units used here.[10] This crease presumably must be due to the significant drop in mean filtration rate ABLE 1), which has been shown to cause increased bicarbonate reabsorption er unit of glomerular filtrate.[10] However, a smaller but statistically signifi-nt increase in bicarbonate reabsorption also occurred following Tris infusion,

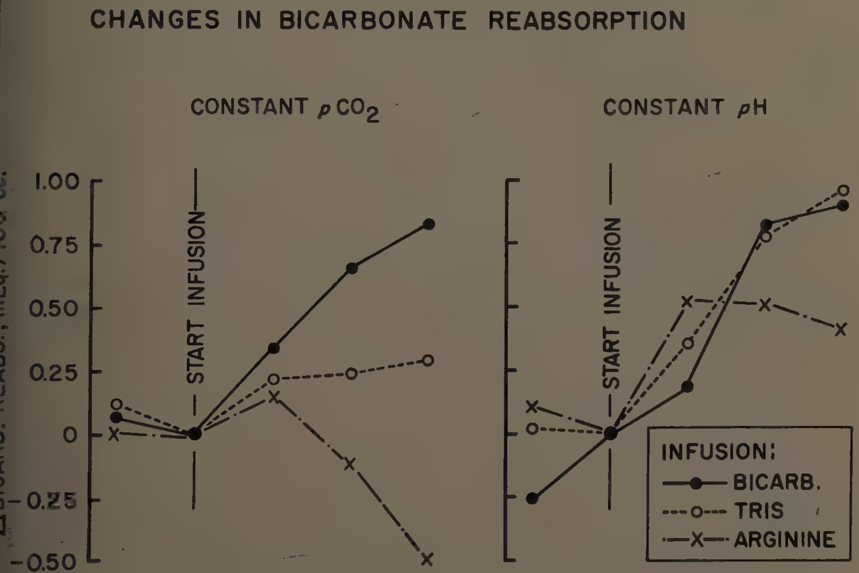


FIGURE 7. Changes in bicarbonate reabsorption per unit glomerular filtrate for constant CO_2 and constant pH experiments.

though there was no drop in filtration rate in this case, suggesting that the tered load of buffer may influence bicarbonate reabsorption independently. nce a drop in filtration rate occurred following arginine infusion, the decrease bicarbonate reabsorption noted in this case can be explained only on the sis of a stimulus to the mechanism of bicarbonate reabsorption on the cellular el, such as a decrease in intracellular pH.

The different renal responses to bicarbonate and organic base infusion cannot definitely explained by our data, but two main possibilities exist:

(1) Hydrogen ion secreted at a constant rate may be increasingly reabsorbed th reabsorbed organic buffer, much as most secreted H^+ is reabsorbed after ction with bicarbonate. This would make the urine more alkaline and de-ease the rate of acid excretion without any primary effect on tubular hydrogen secretion but rather due to an effect on the filtered load of reabsorbed buffer.

Such an explanation would require nearly complete reabsorption of filter buffers, but may be possible under the conditions of our experiment.

(2) The organic compounds, which probably diffuse relatively rapidly in cells, including renal tubule cells, in the un-ionized form would be expected have more effect on intracellular pH than comparable loads of bicarbonate If renal tubule intracellular pH is raised more by organic compound infusio one would expect tubular hydrogen ion secretion and net acid excretion decrease more under these circumstances and bicarbonate reabsorption to less. This explanation is also consistent with the observation that Tris a arginine (which ionize roughly to the same degree at physiological pH) behave very similarly with regard to both acid excretion and bicarbonate reabsorptio while both behaved differently from bicarbonate infusion.

Another theoretically possible mechanism by which decreased acid excretio could be explained would be a decrease in the excreted load of phospha and consequent decrease in titratable acid excretion.[4] However, phospha excretion increased in every case except in the Tris constant pCO$_2$ experiment and the decreased acid excretion could therefore not be explained on this basi Since no consistent differences were noted between the effects of the three typ of infusions on phosphate excretion, such differences could not explain the differences between the organic and inorganic infusions on acid excretion.

It therefore appears that the difference in responses to bicarbonate and the organic compounds might be due either to differences in the filtered load reabsorbed buffer (probably greater in the case of the organic compounds) or differences in their effect on intracellular pH (again greater in the case of organ compounds).

Summary

The effect of bicarbonate, Tris, and arginine infusions on both acid excreti and bicarbonate reabsorption imply that infusions of Tris or arginine decrea acid excretion (or increase base excretion) more than infusions of bicarbona for comparable blood changes, both with and without a superimposed respir tory acidosis. On the basis of this single phenomenon, bicarbonate wou appear to be more suitable for prolonged alkalinization *in vivo*.

The differences in response can be explained either by greater renal reabsor tion of buffer or greater rise in intracellular pH with infusion of the organ compounds. Our data cannot distinguish between these possibilities.

The data of both of the constant pCO$_2$ and constant pH are also consiste with the previous observation that metabolic acid-base disturbances have mo effect on urine pH and acid excretion than do respiratory disturbances of comparable degree.

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EFFECT OF 2-AMINO-2-HYDROXYMETHYL-1,3-PROPANEDIOL ON ELECTROLYTE EXCRETION*

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Nahas and his associates[1] have reported that the administration of 2-amin 2-hydroxymethyl-1,3-propanediol (THAM) to dogs maintained in "apn oxygenation" resulted not only in lowering of the arterial $p\text{CO}_2$ and correcti of respiratory acidosis but also in a marked increase in the urinary loss electrolytes. The present investigation was initiated to study the effect a the mechanism of action of THAM on the renal excretion of electrolytes in t normal dog.

Methods and Procedures

Twelve mongrel dogs weighing between 15 and 18 kg. and anesthetized wi 30 mg./kg. of pentobarbital were employed in this investigation. Standa clearance procedures were employed to study the renal effects of THAM. F this purpose, an infusion of isotonic saline, which contained a sufficient quant of creatinine for the measurement of glomerular filtration rate (GFR), w started at a rate of 5 or 10 ml./min. After the urine flow had stabilized, uri was collected for 2 control clearance periods of 10 to 50 min. each. Sub quently another solution containing varying amounts of THAM, but identi in all other respects to the control infusate, was administered intravenously 5 or 10 ml./min. Following the stabilization of the urine flow, the experime was concluded with the collection of urine in 3 or more clearance periods. the mid-point of each period, blood samples were drawn through an indwelli femoral arterial needle into a heparinized syringe. In a second series of periments, the rate of excretion of C^{14}-labeled THAM was determined. this experiment, 340 ml. of isotonic THAM containing tracer amounts of C labeled THAM (specific activity of 83 μc/mg.) was injected intravenously a the urine collected at intervals during the subsequent 20 hours.

Blood and urine pH were determined anaerobically with a Beckman meter at room temperature and corrected to 37° C. Total CO_2 content blood and urine were determined by the manometric method of Van Slyke a Neill.[2] Concentration of bicarbonate and dissolved CO_2 of the blood and urine were calculated from the Henderson-Hasselbalch equation. Urinary a plasma sodium and potassium level were determined by flame photome using lithium as an internal standard. The osmolal concentration of plasma and urine were determined by the method of freezing point depress employing a Fisk osmometer. The urine and plasma chloride and creatin concentrations were determined by the chemical methods routinely employ our laboratory.[3,4] C^{14}-labeled THAM in the urine was counted with a dowless gas-flow counter.

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* The investigation reported in this paper was supported in part by the United St Army Medical Research and Development Command, Departments of the Army, un Contract DA-49-007-MD-429, Washington, D. C.; by Research Grant H-444-C10 from National Heart Institute, Public Health Service, Bethesda, Md.; and by grants from Massachusetts Heart Association, Boston, Mass., and the Helen Hay Whitney Foundat New York, N.Y.

Results

rine flow. TABLE 1 shows the effect of infusion of THAM on the rate of e flow in a representative experiment. The rate of urine flow increased n 1.4 to 9.3 ml./min. The increase in urine flow occurred within a few

TABLE 1
EFFECT OF INFUSION OF THAM ON URINE FLOW

Time (min.)	V (ml./min.)	Osmolal conc. (mOsm./l.) Plasma	Urine	U/P (osmolal ratio)
sion: 0.85 per cent NaCl 10 ml./min.				
0–20	1.4	308	779	2.5
21–40	1.6	303	672	2.2
sion: 0.3 *M* THAM 10 ml./min.				
95–105	10.5	310	440	1.4
105–115	9.3	315	430	1.3
115–125	9.3	317	436	1.3

TABLE 2
RELATIONSHIP BETWEEN OSMOLAL CLEARANCES, URINE FLOW, AND FREE WATER CLEARANCE FOLLOWING THE INFUSION OF THAM

Time (min.)	V (ml./min.)	Osmolal conc. (mOsm./l.) Plasma	Urine	U/P (osmolal ratio)	C_{osm} (ml./min.)	C_{H_2O} (ml./min.)
0–60	Infusion: 0.85 per cent NaCl 10 ml./min.					
60–70	3.5	333	447	1.3	4.85	−1.35
70–80	3.5	330	470	1.4	4.9	−1.5
80–130	Infusion: 0.3 *M* THAM 10 ml./min. in 0.85 per cent NaCl					
130–140	13.8	343	395	1.0	15.9	−2.1
140–150	14.2	343	400	1.0	16.6	−2.4
150–160	14.6	357	401	1.0	16.4	−1.8

utes and reached a maximal rate about 1 hour after the infusion of THAM. s apparent that the plasma-osmolal concentration did not change appreci- y, but the urinary osmolal concentration dropped from 779 to 436 mOsm./l. a corresponding fall in U/P (urine/plasma) osmolal ratio to 1.3. The tionship between the urine flow (V), plasma and urine osmolality, osmolal rance (C_{osm}), and free water clearance (C_{H_2O}) following the infusion of AM are summarized from another experiment in TABLE 2.

he effect of THAM on the urinary excretion of sodium, potassium, and ride appears in TABLE 3. It is seen that GFR and the plasma concentra- s of these electrolytes change very slightly. However, in similar experiments in which a larger quantity of THAM was infused, the plasma concent tion of these electrolytes fell significantly. The renal excretion of sodiu potassium, and chloride increased markedly in all of our experiments. T percentage of the filtered load of sodium excreted increased from 2.6 to 10.5 cent, that of chloride from 3.5 to 10.7 per cent, and that of potassium from 1 to 100 per cent. In several clearance periods in 2 dogs more potassium v excreted in the urine than could be accounted for by the calculated filtered lo of this ion, thus suggesting that the administration of THAM may result tubular secretion of potassium. The effect of increasing concentration THAM on the renal excretion of electrolytes was studied by consecutive ministration of solutions containing 0.075, 0.15, 0.30, and 0.60 *M* THAM a constant rate of infusion. The result of one such experiment appears TABLE 4. It is evident that infusion of increasing concentration of THA

Table 3

EFFECT OF INFUSION OF THAM ON URINARY EXCRETION OF ELECTROLYTES

Time (min.)	V (ml./min.)	GFR (ml./min.)	Plasma conc. (mEq./l.)			Excretion (μEq./min.)			Filtrate excre (%)		
			Na	K	Cl	Na	K	Cl	Na	K	
Infusion: 0.85 per cent NaCl 10 ml./min.											
0–20	1.4	85	155	4.2	127	384	59	308	2.6	18.4	
20–40	1.6	85	155	4.4	127	384	67	342	3.0	18.0	
Infusion: 0.3 *M* THAM in 0.85 per cent NaCl 10 ml./min.											
95–105	10.5	89	145	4.2	116	1291	357	1350	11.5	95	1
105–115	9.3	85	148	4.2	121	1442	363	1190	11.5	101	1
115–125	9.3	83	151	4.5	120	1330	372	1070	10.5	100	1

results in a step-wise increase in the rate of urine flow and the urinary cretion of electrolytes.

As reported by several other investigators, the infusion of THAM in experiments invariably produced a significant elevation of blood and urine and a marked increase in the urinary excretion of bicarbonate. The detai data from a representative experiment appear in TABLE 5. It is evident t the blood *p*H increased from an initial level of 7.27 to 7.42 and 7.53 follow the infusion of 459 ml. of 0.30 and 300 ml. of 0.60 *M* THAM respectively. urine *p*H showed a similar but more striking rise from 5.82 to 7.61 during period. Total CO_2 content of the blood increased from 18.8 to 31.2 mmoles and the plasma bicarbonate level rose from 17.6 to 30 mmoles/l. The urin pCO_2 showed an initial increase followed by a subsequent fall to the ini control level. The plasma pCO_2, however, remained essentially const The urinary excretion of bicarbonate increased from 1.3 to 927 μmoles/m and its tubular reabsorption diminished from 1142 to 528 μmoles/min. excretion of bicarbonate increased from 0.1 to 64 per cent of the filtered lo

The renal excretion of THAM was studied after the rapid administratio

0 ml. of isotonic THAM solution containing tracer amounts of C^{14}-labeled HAM. FIGURE 1 depicts the per cent of injected C^{14} as count per minute pearing in the urine during a 20-hour period. It is evident that about 25 r cent of the injected C^{14} could be recovered in the urine within the first hour. uring the subsequent 18 hours only an additional 20 per cent of the total C^{14} jected appeared in the urine.

TABLE 4

EFFECTS OF INFUSION OF INCREASING CONCENTRATION OF THAM ON URINE FLOW AND ELECTROLYTE EXCRETION

Time (min.)	V (ml./min.)	GFR (ml./min.)	Plasma conc. (mEq./L.)			Excretion (μEq./min.)			Filtrate excreted (%)		
			Na	K	Cl	Na	K	Cl	Na	K	Cl
0–130	Infusion: 0.85 per cent NaCl 5 ml./min.										
71–100	0.23	65	155	4.2	121	9.2	6.9	30	0.1	2.5	0.4
101–130	0.25	65	153	3.8	122	10.0	8.0	29	0.1	3.3	0.4
131–165	Infusion: 0.075 *M* THAM 5 ml./min.										
165–175	2.0	66	150	3.0	120	192	72	388	1.9	36.5	4.9
175–185	2.0	66	150	3.0	122	184	76	372	1.9	38.5	4.6
185–215	Infusion: 0.15 *M* THAM 5 ml./min.										
215–225	3.8	76	146	2.8	117	403	123	572	3.7	58	6.5
225–235	3.5	70	146	3.0	117	372	105	510	3.7	50	6.2
235–265	Infusion: 0.3 THAM buffer 5 ml./min.										
265–275	7.0	69	145	3.0	115	685	112	735	6.8	54	9.3
275–285	7.0	70	143	2.6	115	685	112	765	6.8	61	9.4
285–300	Infusion: 0.6 *M* THAM 5 ml./min.										
300–310	16.2	76	139	3.4	115	1540	194	1540	14.5	75	17.7
310–320	16.0	71	140	3.0	113	1600	160	1600	16.0	75	19.8

TABLE 5

EFFECT OF INFUSION OF THAM ON RENAL EXCRETION OF BICARBONATE

Time (min.)	V (ml./min.)	GFR (ml./min.)	Blood				Urine			$BHCO_3$		
			*p*H	CO_2 (mM/l.)	*p*CO_2 (mm. Hg)	$BHCO_3$ (mM/l.)	*p*H	CO_2 (mM/l.)	*p*CO_2 (mm. Hg)	Exc. (μEq./min.)	Reab. (μEq./min.)	Filt. Exc. (%)
0–150	Infusion: 0.85 per cent NaCl 5 ml./min.											
0–120 0–150	0.5	65	7.27	18.8	39.9	17.6	5.82	26.4	100	1.3	1142	0.1
0–240	Infusion: 0.3 THAM 5 ml./min.											
0–220 0–230	4.5	56	7.42	26.6	40.3	25.6	7.43	112	162	482	948	34
0–300	Infusion: 0.6 THAM 5 ml./min.											
0–280	8.9	49	7.53	31.2	37	30	7.61	106	103	927	528	64

Discussion

Several investigators have reported the diuretic property of THAM in th dog. Nahas has noted that the infusion of THAM to dogs maintained i "apneic oxygenation" produced a "marked" diuresis.[1] Our studies confirm th diuretic property of THAM in the normal dog. Diuresis does not appear to b related to an increase in the rate of glomerular filtration. The latter was qui variable from one experiment to another and did not show a uniform patter

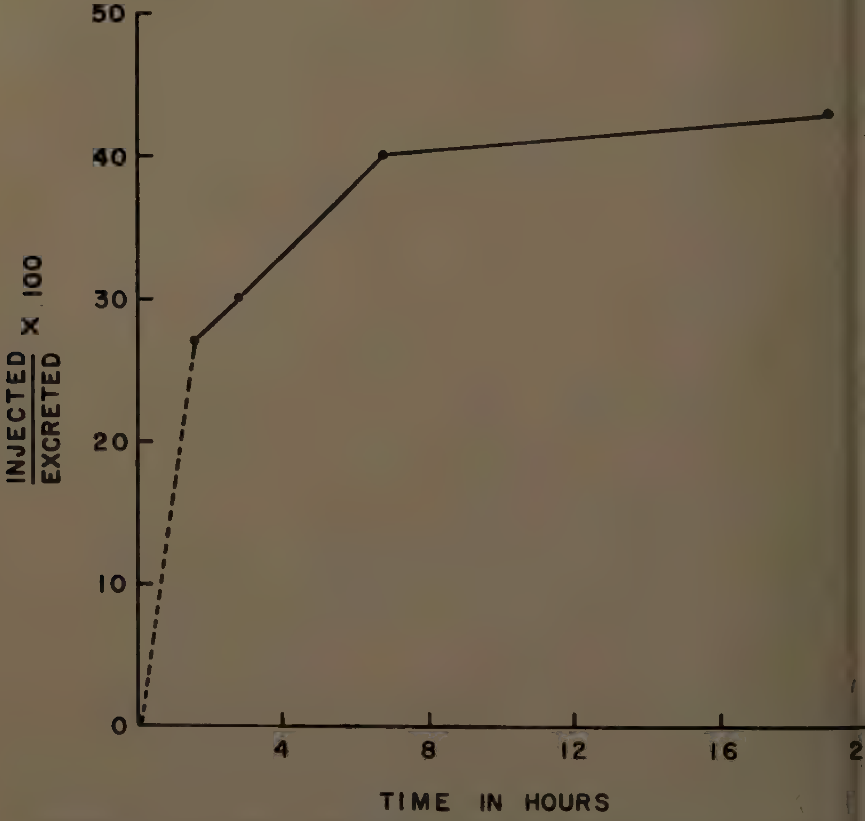
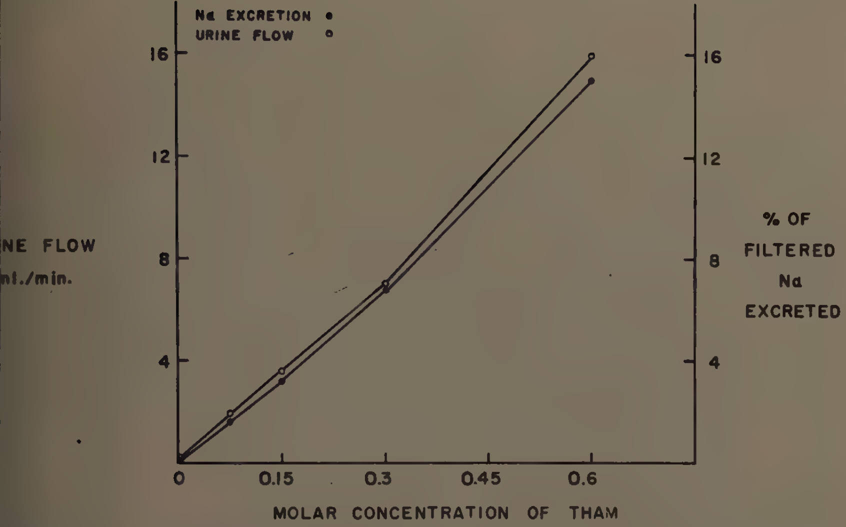
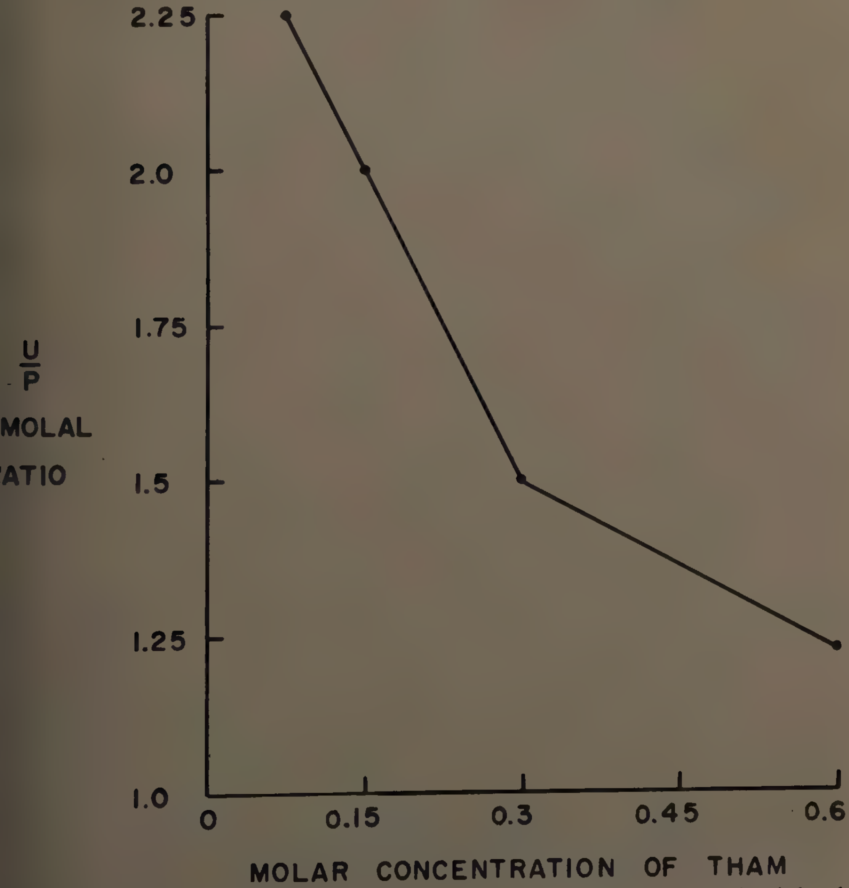


FIGURE 1. The excretion of C^{14}-labeled THAM in the urine following the administrati of 340 ml. of THAM containing tracer amounts of C^{14}-labeled THAM.

As shown in FIGURE 2, the rate of urine flow paralleled the urinary excretion sodium. Moreover, the rate of urine flow and the urinary excretion of sodiu increased more or less in direct proportion to the molar concentration of THA infused. FIGURE 3 depicts the relationship between the osmolal concentrati of THAM and the U/P osmolal ratio. It is apparent that with increasi concentration of THAM the U/P osmolal ratio fell and approached unit These observations are consistent in general with the findings in osmo diuresis[5,6] and suggest that THAM behaves, at least in part, as an osmo diuretic.



IGURE 2. The relationship between the urine flow, renal excretion of sodium, and the lar concentration of THAM being infused.



IGURE 3. The relationship between the molar concentration of THAM being infused the U/P osmolal ratio.

However, there are at least several lines of evidence indicating that the re effects of THAM are not mediated merely through its osmotic effect. Nah and his associates have shown that after administration of THAM only 60 cent of THAM appears in the urine over the subsequent 72-hour period. F thermore, our studies with C^{14}-labeled THAM show that only a small p centage of C^{14} appears in the urine following the injection of C^{14}-labeled THA In order to produce an effective diuresis, it would be expected that a lar quantity of THAM be excreted more rapidly in the urine. Furthermore, it h been repeatedly demonstrated that during osmotic diuresis a considera fraction (more than 50 per cent) of filtered water could be excreted with onl slight increase in bicarbonate excretion.[7] Therefore, the disproportionat greater increase in the urinary excretion of bicarbonate as compared to that water, sodium, and chloride cannot be explained merely on the basis of ministration of an osmotically active agent. As will be discussed later, it likely that THAM probably exerts some specific effect on the renal tubu

In all of our experiments the administration of THAM resulted in a mark increase in the urinary excretion of bicarbonate that was accompanied by o a moderate rise in plasma bicarbonate level. However, in the presence of unchanged or slightly reduced GFR, the filtered load of bicarbonate increa only slightly. The increase in the urinary excretion of bicarbonate can therefore be attributed to an increase in the filtered load of bicarbonate a must be a consequence of a diminution in tubular reabsorption of bicarbona Indeed, our data support this contention by showing that the tubular rea sorption of bicarbonate decreases following the administration of THAM fr the control level of 99 per cent to 44 per cent of the filtered load.

It is generally agreed that the renal tubular reabsorption of bicarbonate accomplished by means of (H^+)-(Na^+) exchange mechanism.[8,9] Any proc that interferes with this mechanism will diminish the tubular reabsorption a increase the urinary excretion of bicarbonate. One such process is a reduct in H^+ concentration of the renal tubules. The diminution in intracellular concentration could result either from a reduction in the formation of H^+, as the inhibition of carbonic anhydrase (C.A.) activity or from the intracellu binding of H^+. There is evidence that THAM has no effect on C.A. activity *vitro*. Furthermore, the marked increase in excretion of bicarbonate is sign cantly greater than that obtained following the administration of a C.A. hibitor. The injection of a maximum dose of acetazalomide in the dog resu in an excretion of about 30 per cent of the filtered load of bicarbonate[10,11] compared to 50 to 60 per cent noted following the administration of THA In addition, the marked increase in chloride excretion that approximates sodi excretion is not consistent with a carbonic anhydrase inhibition effect.

Our data are consistent with the hypothesis that the administration of TH binds the intracellular H^+, thus making the latter unavailable for (H^+)-(N exchange. Potassium is subsequently secreted by the renal tubules to repl H^+ in the distal tubular (H^+)-(Na^+) exchange mechanism. This hypothe however, assumes that THAM penetrates the cell. Several preliminary st ies with C^{14}-labeled THAM have shown a larger volume of distribution

AM than that for inulin, thus suggesting intracellular penetration of AM.*

n conclusion we suggest the scheme for the renal action of THAM as shown FIGURE 4. Following the administration of THAM, a relatively small tion is excreted in the urine whereby it might exert some osmotic effect 1 a resultant increase in the urine flow and in the urinary excretion of son and chloride. The bulk of available data, however, suggests that THAM bably enters the cell where it buffers intracellular H^+. In the case of the al tubules, the diminution in the tubular H^+ concentration results in a dease of (H^+)-(Na^+) exchange. As a consequence K^+ is secreted by the renal ules to replace H^+ in the (Na^+)-(H^+) exchange scheme, thus leading to an

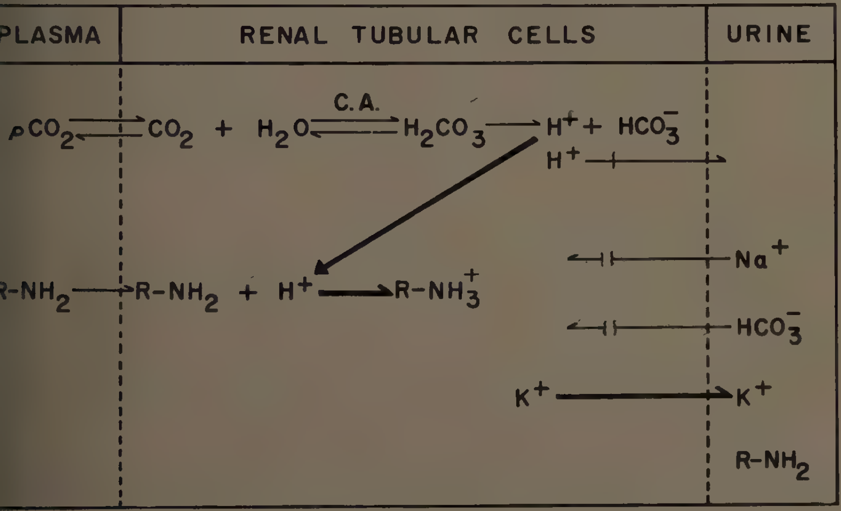


FIGURE 4. The scheme of mechanism of renal action of THAM.

ease in urinary excretion and occasionally in secretion of potassium and a ked rise in the urinary excretion of bicarbonate.

Acknowledgment

he THAM and C^{14}-labeled THAM used in these experiments were genısly supplied by Abbotts Laboratories, Chicago, Ill.

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FECTS OF 2-AMINO-2-HYDROXYMETHYL-1,3-PROPANEDIOL ON RENAL FUNCTION IN REFERENCE TO SALICYLATE AND GLUCOSE[*]

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-Amino-2-hydroxymethyl-1,3-propanediol or Tris(hydroxymethyl)amino- thane (THAM), an organic buffer of low toxicity, has been widely used perimentally for correction of metabolic and respiratory acidosis.[1,2,3] It was wn that this compound exerted a marked diuretic effect with production of aline urine. It was reported that its intravenous administration in pan- atectomized or phloridzinized animals[4a, b] was accompanied by a marked de- ase in glucosuria. The present series of experiments were designed to deter- ne the significance of the changes produced by THAM administration in the retion of salicylates and in the induced glucosuria.

EFFECT OF THAM ON ACUTE SALICYLATE INTOXICATION

Methods and Results

'our mongrel dogs were studied. A detailed description of the methods and ults was made in a previous publication.[5] A single dose of 100 mg./kg. of lium salicylate was injected intravenously into the animals. Thirty min. er a 0.3 *M* THAM solution was administered intravenously for 30 min. at the e of 1 ml./kg./min. for the first 15 min. and at the rate of 0.5 ml./kg./min. the last 15 min. This amounts to a total dose of 660 mg./kg. Thirty min. owing onset of THAM infusion, salicylate excretion in the urine increased m 723 to 2,838 μg./min., a 4-fold increase, while pH of the urine rose from 4 to 7.59 (FIGURE 1). There was a 10-fold increase in HCO_3^- excretion l Na^+ rose from 413 to 477 μEq/min. and K^+ from 47 to 131 μEq/min. loride excretion decreased from 1,030 to 865 μEq/min. (FIGURE 2). There s a marked diuresis with no significant changes in inulin or PAH clearance. enty-five min. after THAM administration, plasma salicylate levels had pped from 33 to 17 mg./100 ml. (FIGURE 1). The highest blood pH was 7, CO_2 rose from 23.6 to 32.5 mM/l. HCO_3^- from 22 to 31 mM/l., and pCO_2 m 32.5 to 36.6 mm. Hg (FIGURE 3). Na^+, K^+ and Cl^- in plasma fell slightly consistently (FIGURE 2).

Discussion

Vhen THAM was used in the treatment of experimentally produced acute cylate intoxication, a significant increase in salicyluria was observed. This accompanied by a marked increase in plasma bicarbonate. Regardless of final proof of the mechanism by which salicylate is reabsorbed or secreted, present experiments indicate that both an alkaline urine and an increase in

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[*] The work described in this paper was supported in part by Research Grant H-4859 from National Heart Institute and Research Grant A-4359 from the National Institute of hritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

urine formation enhance the elimination of salicylate by the kidney, as pr
viously emphasized by others.[6,7,8,9] The relative importance of these two fa
tors, alkalinization of the urine and diuresis, in the elimination of salicylate
still to be determined. Furthermore, protein-binding of salicylate is *p*H d

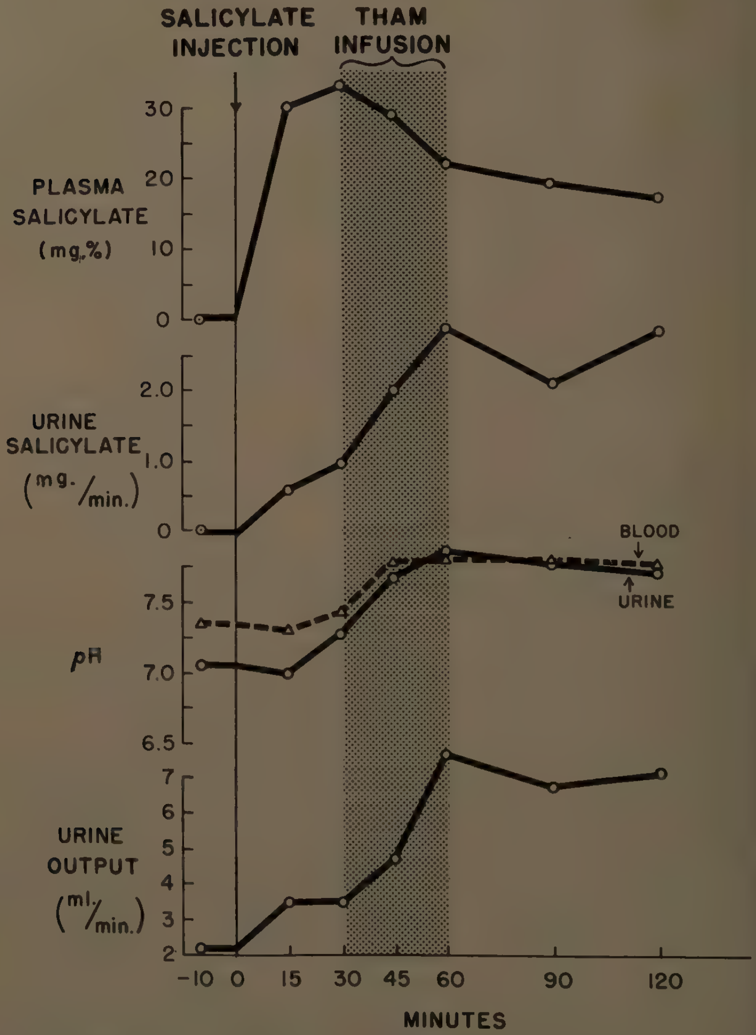

FIGURE 1. Effects of I.V. administration of THAM (0.3 *M* solution, 1 ml./kg./min.
30 min., total dose 660 mg./kg.) on plasma salicylate levels, urine salicylate excretion, ur
output, and blood and urine *p*H in 4 dogs injected with 100 mg./kg. of sodium salicylate.

pendent; the lower the *p*H, the greater the binding. It is therefore importa
to have an alkalinization of body fluids so that this binding be minim
THAM will produce such alkalosis in intracellular[10] as well as extracellu
fluid and may therefore limit the protein-binding of salicylate and favor

al excretion. In addition, THAM has also protein-binding properties its own as suggested by Hyman Rosen and Gabriel G. Nahas (unpublished a) and may compete with salicylate for such binding. All these properties THAM (buffering, diuretic, and protein-binding) contribute to make it a

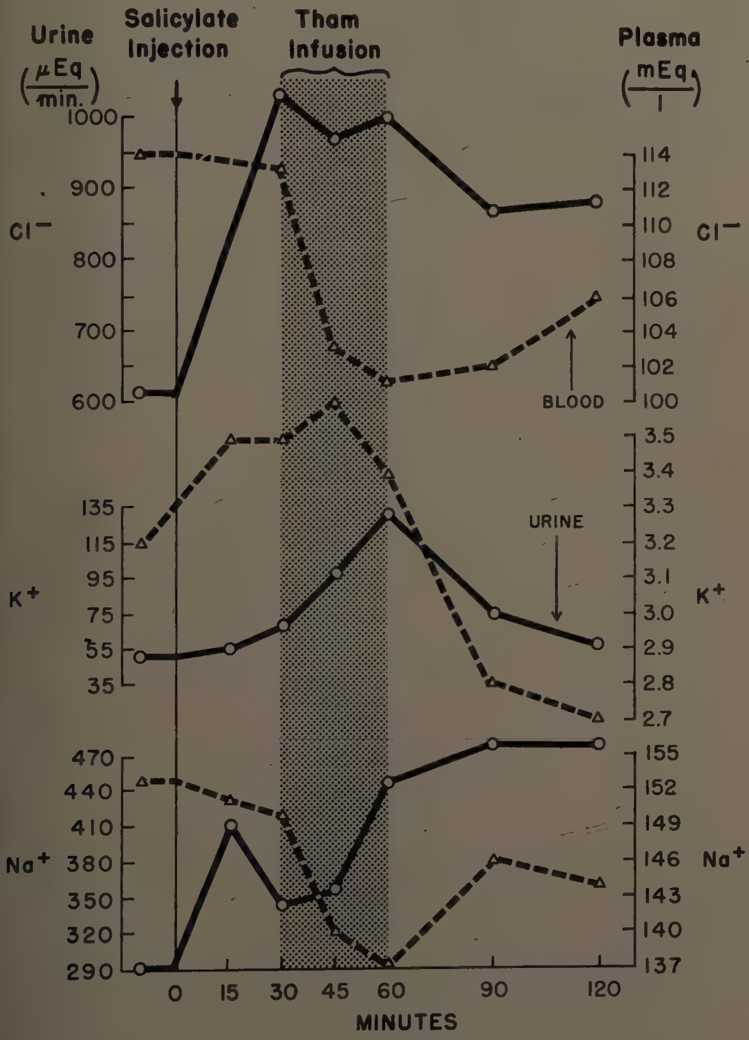

FIGURE 2. Effects of I.V. administration of THAM (0.3 *M* solution, 1 ml./kg./min. for 30 ., total dose 660 mg./kg.) on plasma Na^+, Cl^-, and K^+ levels, and on urinary excretion he same electrolytes in 4 dogs injected with 100 mg./kg. of sodium salicylate.

ry effective agent in acute salicylate poisoning. The depression of ventila- n that accompanies THAM administration was much less marked in the esent series of experiments. During the period of treatment with THAM, erage $PaCO_2$ did not rise above 36.6 mm. Hg, which is 7 mm. Hg below the control value, indicating that THAM did not completely suppress the stim lating effect of salicylate on ventilation.

Since THAM has been found to be safe[3,11,12] in doses similar to those used the present experiments, it should be beneficial in treatment of infants and ch dren suffering from salicylate poisoning. THAM has already been used in c such instance.[13]

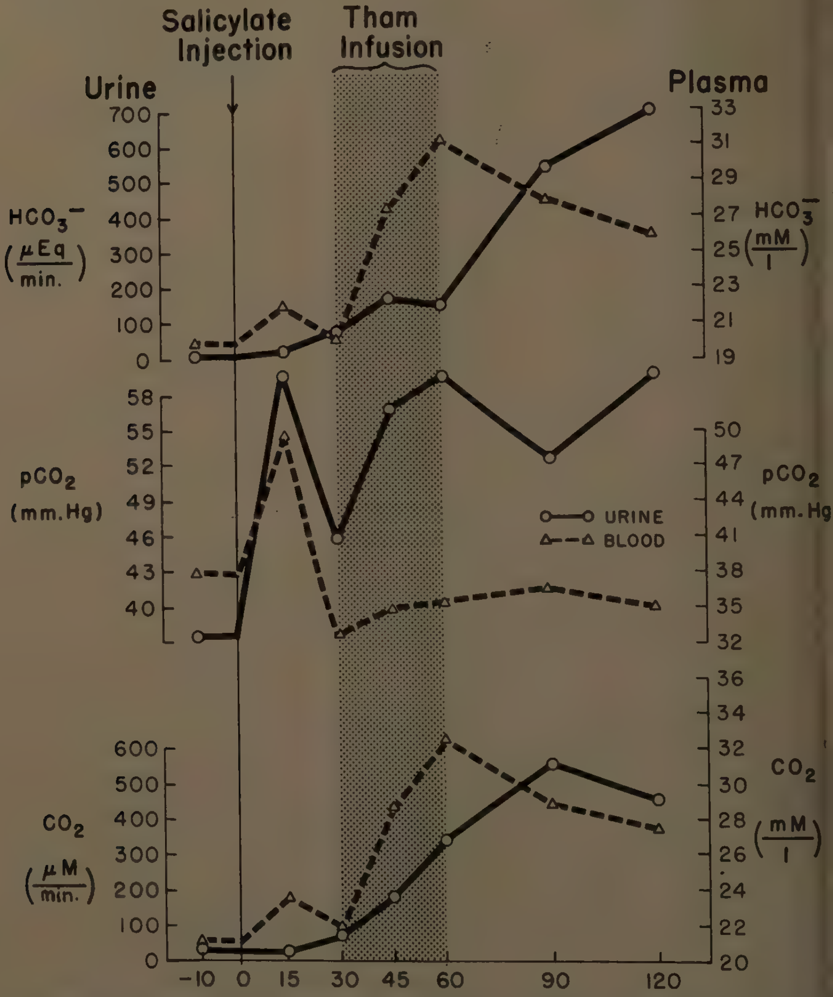

FIGURE 3. Effects of I.V. administration of THAM (0.3 *M* solution, 1 ml./kg./min. 30 min., total dose 660 mg./kg.) on HCO_3^- levels in plasma, urinary HCO_3^- excret pCO_2, total plasma CO_2, and urinary CO_2 excretion in 4 dogs injected with 100 mg./kg sodium salicylate. The analyses at 15 min. were done on one dog.

EFFECT OF THAM ON INDUCED GLUCOSURIA

Method

Two series of experiments were performed. In the first series, 3 mong dogs weighing 26 to 28 kg. were pancreatectomized and treated with penici omycin, and insulin for 4 to 6 days. For 24 hours before the experiment, ogs were not fed, but were allowed to drink water *ad libitum*, and no n was administered. From then on, the experimental protocol was the as the one used in the salicylate experiments,[5] with the difference that studies were not made. After a control period, an infusion of 0.3 *M* M was administered, at a rate close to 1 ml./kg./min. for 60 min. The ge total dose was 2.0 to 2.2 gm./kg. In the second series, 6 mongrel dogs, ing 16 to 19 kg. and unfed for 24 hours but given water *ad libitum*, were At the end of two 10-min. periods of clearance, they received simul-usly 50 mg. phloridzin/kg. body wt. in 2.5 per cent sodium bicarbonate nd 50 mg./kg. body wt. in propylene glycol subcutaneously, so as to en-

Table 1

T of THAM Administration* on Glucose Plasma Levels, Glucosuria, and Urine Volume of Three Pancreatectomized Dogs

Time (min.)	Glucose mg./100 ml.		Urine volume (ml./min.)
	Plasma	Urine	
15	366 (348–384)†	7505 (4000–11010)	1.39 (1.0–1.78)
30	359 (342–376)	5995 (2750–9240)	2.31 (1.56–3.06)
60	338.5 (314–363)	985 (650–1320)	7.76 (7.21–8.3)
90	319.5 (316–323)	166 (165–167)	9.65 (8.7–10.6)
120	318.5 (298–339)	74.5 (55–94)	4.41 (1.5–7.31)
150	316.5 (314–319)	22 (13–31)	3.52 (2.14–4.9)

V., 0.3 *M*, 1 ml./kg./min.
ange.

rapid and prolonged action of the drug. Two urine collections of 30 min. were performed and followed by an infusion of THAM at the rate of 1 ml./ in. for 60 min. There were two additional urine collections during this r period and another collection in the first hour following the discontinua-f THAM.

Results

ncreatectomized dogs (*Group 1*). At the end of THAM infusion, glucose in ine fell from 5995–7505 to 22.0–74.5 mg. per cent while blood glucose only om 359–366 to 316–318 mg. per cent. Urinary output reached a maxi-of 9.6 ml./min. at 90 min. and then fell to 4 ml./min. at 120 to 150 min. E 1 and Figure 4). Inulin clearance fell from 108–130 ml./min. to 55 ml./ a 55 per cent decrease, reaching its lowest point 120 min. after the start of the experiment and 30 min. after discontinuation of THAM (TABLE 2). T were no marked changes in arterial blood pressure during THAM adminis tion; however, after the end of the infusion, it decreased significantly. Si taneously, glucose clearance fell from 31.5–33.7 ml./min. to 5 ml./min., a

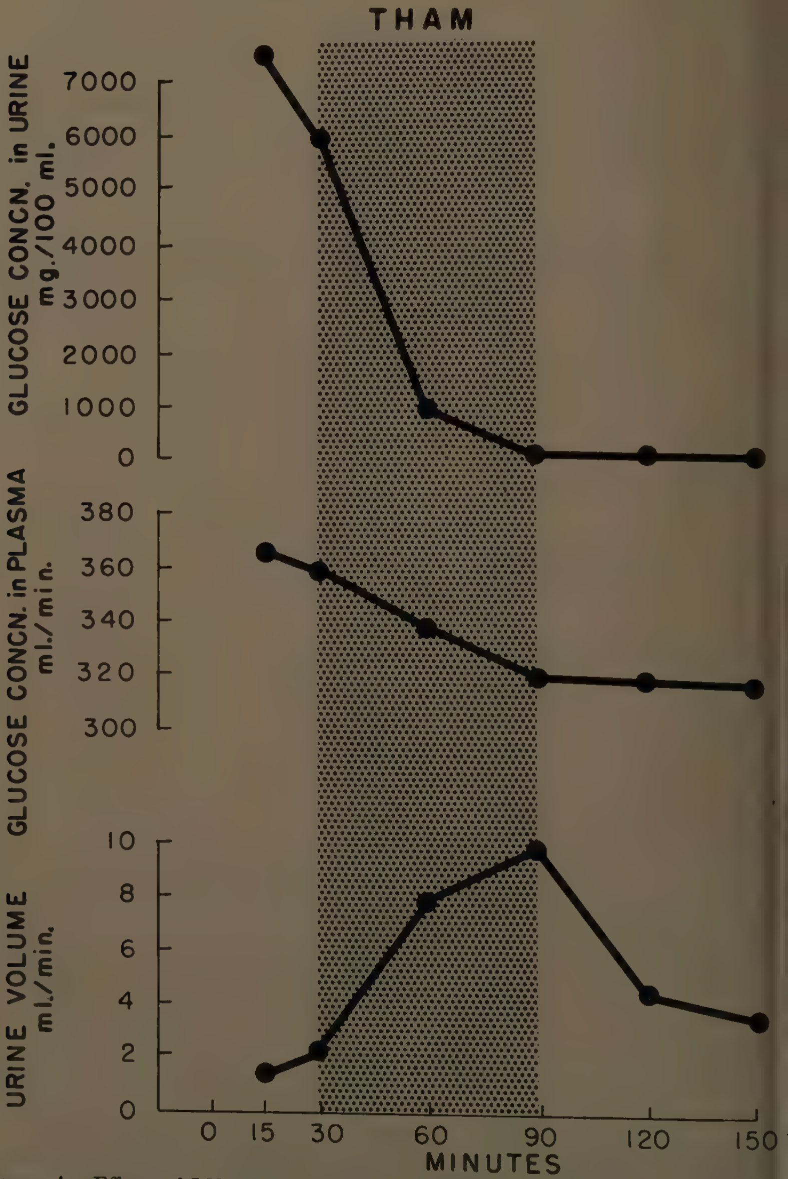

FIGURE 4. Effects of I.V. administration of THAM (0.3 *M* solution, 1 ml./kg./min 60 min., total dose 2 to 2.2 gm./kg.) on glucose concentration in urine and plasma, and nary volume in 3 pancreatectomized dogs.

TABLE 2

EFFECTS OF THAM ADMINISTRATION* ON INULIN CLEARANCE, GLUCOSE CLEARANCE, GLUCOSE CLEARANCE/INULIN CLEARANCE RATIO, TUBULAR REABSORPTION OF GLUCOSE, GLUCOSE LOAD FILTERED AT THE GLOMERULI, PERCENTAGE OF THE FILTERED LOAD OF GLUCOSE REABSORBED,† AND ARTERIAL BLOOD PRESSURE OF THREE PANCREATECTOMIZED DOGS

Time (min.)	C_{In} (ml./min.)	C_G (ml./min.)	C_G/C_F	T_G (mg./min.)	P_GC_F (mg./min.)	$(T_G/P_GC_F) \times 100$ (%)	Art. Bl. Press. mm. Hg
15	130.6 (87.3–174)‡	33.7 (10.4–56.9)	0.22 (0.12–0.33)	352.3 (295–409.5)	470 (335.0–605.5)	78 (68–88)	199/101 (185/89–213/113)
30	108.6 (87.3–130)	31.5 (24.6–38.4)	0.29 (0.28–0.30)	279.2 (213.7–344.7)	394 (298.4–490.7)	72 (71–72)	183/92 (173/84–193/100)
THAM 60	114.5 (61–168)	21.9 (17.4–26.3)	0.22 (0.16–0.28)	326.8 (139–514.7)	401 (191.5–609.8)	79 (73–84)	186/98 (185/96–187/100)
90	56.6 (36–77)	5.0 (4.7–5.4)	0.11 (0.06–0.15)	163.8 (98.8–228.8)	180 (116.3–243.6)	90 (85–94)	186/92 (167/87–205/96)
120	19.4 (11–27.6)	1.2 (0.28–2.0)	0.49 (0.03–0.73)	59.3 (31.9–86.6)	63 (33.1–93.5)	95 (93–97)	173/108 (160/126–185/89)
150	30.6 (12.2–49)	0.21 (0.2–0.21)	0.01 (0.004–0.017)	97.2 (38–156)	97 (38.3–156.3)	100 (99.4–99.9)	147/78 (120/60–173/96)

Key: inulin clearance, C_F ; glucose clearance, C_G ; glucose clearance/inulin clearance ratio, C_G/C_F ; tubular reabsorption of glucose, T_G ; glucose load filtered at the glomeruli, P_GC_F.

* I.V., 0.3 *M*, 1 ml./kg./min.

† Represented by $T_G/P_GC_F \times 100$.

‡ Range.

crease of 85 per cent, and was 0.21 ml./min. at the end of the experim (TABLE 2). To appreciate the changes in glucose clearance due to inulin cl ance, the C_G/C_F ratio was calculated. It decreased from 0.22–0.29 to 0.11 the end of THAM administration. A greater decrease was observed in

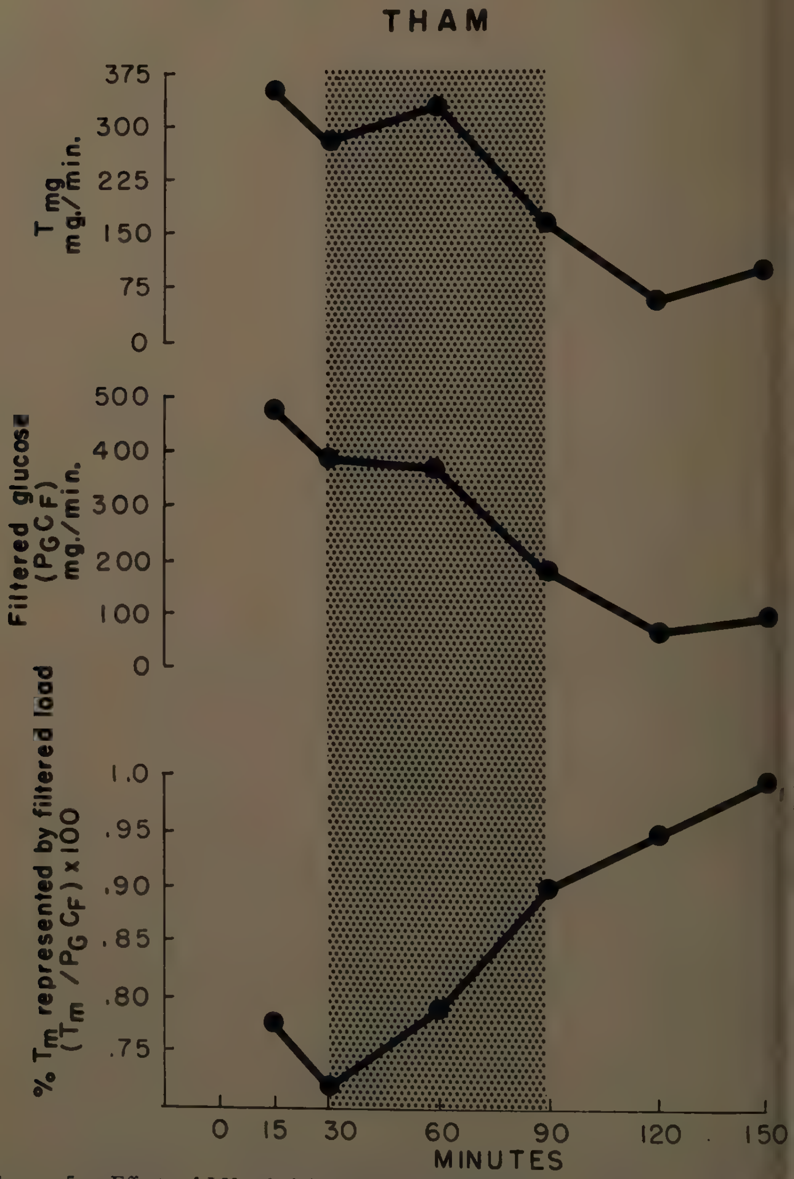

FIGURE 5. Effects of I.V. administration of THAM (0.3 *M* solution, 1 ml./kg./min 60 min., total dose 2 to 2.2 gm./kg.) on tubular reabsorption of glucose, filtered loa glucose (P_GC_F), and tubular reabsorption of glucose expressed as percentage of the filt load of glucose (T_G/P_GC_F × 100).

lar reabsorption of glucose or T_G from 279–352 mg./min. to 163 mg./min.
) min. and to 59 mg./min. at 120 min. after the beginning of the experiment
LE 2 and FIGURE 5). The glucose filtered at the glomeruli (P_GC_F) dropped
394–470 mg./min. to 180 mg./min. at 90 min. (end of THAM infusion)
to 63 mg./min. at 120 min. (TABLE 2). Since there was a constant decrease
ne filtered glucose and a greater drop of the T_G, the percentage of the fil-
l glucose (P_GC_F) reabsorbed by the tubule (T_G/P_GC_F) × 100 was calcu-
l. There was a constant increase in the percentage of glucose reabsorbed,
72–78 to 90 at 90 min. and 100 at 150 min. (TABLE 2 and FIGURE 5).
re was no correlation between glucose reabsorption and bicarbonate clear-
or *p*H and CO_2 of blood or urine.

TABLE 3

CT OF THAM ADMINISTRATION* ON URINE/PLASMA RATIO OF INULIN, GLUCOSE, AND TOTAL OSMOLALITY OF THREE PANCREATECTOMIZED DOGS

	Time (min.)	U/P Inulin	U/P Glucose	U/P Osmolar
	15	68.8 (39.7–97.8)†	21.0 (10.4–31.6)	3.1 (1.8–4.4)
	30	62.5 (41.8–83.2)	16.3 (8.0–24.6)	3.1 (1.8–4.4)
M	60	15.3 (7.4–23.3)	2.9 (2.1–3.6)	1.8 (1.7–1.9)
	90	6.2 (3.4–8.9)	0.52 (0.51–0.53)	1.3 (1.1–1.4)
	120	5.6 (3.9–7.4)	0.23 (0.18–0.28)	1.2 (1.1–1.3)
	150	8.5 (7.1–10.0)	0.07 (0.04–0.10)	1.2 (1.1–1.3)

.V., 0.3 *M* 1 ml./kg./min.
Range.

evaluating the reabsorption of water, the different U/P ratios were cal-
ed. There was a marked decrease in the U/P ratio of inulin and glucose.
U/P ratio of total osmolality dropped from 1 to 1.2–1.8 (TABLE 3).
ne solute-free water clearance (C_{H_2O}) was constantly negative. At the
of THAM administration, the pH in blood increased from 7.28 to 7.62 and
ine from 6.06–6.21 to 7.17–7.60. HCO_3^- in blood rose from 17.15–17.77
5.04–30.45 mM/l. and, in urine, from 3.28–3.89 to 20.54–60.88 mM/l.
loridzinized dogs (*Group 2*). The U/P ratios of inulin, glucose, and total
olality were similar to those of the pancreatectomized animals. At the end
HAM administration (TABLE 4) urine volume increased from 4 to 20 ml./
while arterial blood pressures increased slightly. In experiments 7, 8, and
here was an increase in glucose tubular reabsorption (T_G) although it never
ned to control level (TABLE 5). TABLE 6 shows in 5 of 6 experiments a
ant decrease in the filtered glucose load (P_GC_F). TABLE 7 presents the

glucose clearance (C_G), which did not follow any consistent pattern. Inu clearance (C_{In}) (TABLE 8) remained stable. TABLE 9 tabulates the ratio the clearance of glucose over the clearance of inulin (C_G/C_F). The maxim value of this ratio is 1.0, and it indicates a complete lack of tubular reabsorpti of the filtered glucose. A value of 0.9 to 1.0 was reached only before or af the administration of THAM. TABLE 10 shows the constant increase in blo

TABLE 4

EFFECT OF THAM ADMINISTRATION* ON ARTERIAL BLOOD PRESSURE AND URINE VOLU OF SIX PHLORIDZINIZED DOGS

	Time (min.)	Art. blood press. mm. Hg (sist./diast.)	Urine volume (ml./min.)
	15	229/110 (218/100–240/120)†	2.81 (1.04–5.65)
Phloridzin‡	30	183/104.5 (166/84–200/125)	4.35 (2.06–7.85)
	60	197.5/116 (185/102–210/130)	4.08 (2.47–7.37)
	90	197.5/119.5 (192/119–203/120)	4.39 (2.94–8.25)
THAM	120	206/113 (192/102–210/124)	14.65 (11.9–22.0)
	150	226/132.5 (196/124–256/141)	19.73 (8.38–27.8)
	180	146/110.5 (144/119–148/102)	11.42 (4.32–21.7)

* I.V., 0.3 *M*, 1 ml./kg./min.
† Range.
‡ Injection immediately after 30 min. sample.

TABLE 5

EFFECT OF THAM ADMINISTRATION* ON TUBULAR REABSORPTION OF GLUCOSE OF PHLORIDZINIZED DOGS

(T_G) mg./min.

Time (min.)	11†	6	10	8	7	13
15	42.0	54.8	71.2	102.4	176.7	8.4
30	43.9	62.2	75.4	86.8	136.7	10.1
Phloridzin‡						
60	0	12.6	0	46.2	53.0	0
90	—	8.4	0	26.6	7.5	0
THAM 120	19.8	14.9	0	19.3	31.1	0
150	8.9	6.9	0	90.7	15.5	0
180	0	0	0	62.6	13.1	0

* I.V., 0.3 *M*, 1 ml./kg./min.
† Dog number.
‡ Injection immediately after 30 min. sample.

TABLE 6

CT OF THAM ADMINISTRATION* ON GLUCOSE LOAD FILTERED AT THE GLOMERULI OF SIX PHLORIDZINIZED DOGS

$P_G \times C_F$
(mg./min.)

Time (min.)	11†	6	10	8	7	13
15	42.0	55.8	71.3	102.8	176.9	84.0
30	44.0	62.7	75.5	86.7	143.2	102.6
ridzin‡						
60	31.2	43.4	54.2	98.5	155.0	90.6
90	—	52.4	49.6	100.8	91.5	50.5
M{120	28.2	40.9	32.4	75.3	96.7	41.6
150	22.2	40.7	34.2	115.4	55.5	43.3
180	5.9	42.1	22.1	94.4	33.2	46.5

I.V., 0.3 *M*, 1 ml./kg./min.
Dog number.
Injection immediately after 30 min. sample.

TABLE 7

EFFECT OF THAM ADMINISTRATION* ON GLUCOSE CLEARANCE OF SIX PHLORIDZINIZED DOGS

(C_G ml./min.)

Time (min.)	11†	6	10	8	7	13
15	0	0	0.10	0	0.10	0
30	0	0	0	0	0	0
ridzin‡						
60	36.1	32.5	12.1	44.6	66.1	13.6
90	—	38.6	21.3	65.1	79.2	19.8
M{120	16.5	37.5	22.1	62.0	74.0	38.9
150	22.2	41.1	10.1	21.6	77.0	32.1
180	16.8	57.0	19.5	28.2	25.8	20.3

I.V., 0.3 *M*, 1 ml./kg./min.
Dog number.
Injection immediately after 30 min. sample.

TABLE 8

EFFECT OF THAM ADMINISTRATION* ON INULIN CLEARANCE OF SIX PHLORIDZINIZED DOGS

(C_{In}) ml/min.

Time (min.)	11†	6	10	8	7	13
15	49.9	43.6	73.5	78.5	132.4	127.4
30	49.9	46.1	64.1	75.4	92.6	136.9
idzin‡						
60	36.1	45.7	58.0	83.5	101.0	103.0
90	—	46.0	68.0	88.4	86.5	81.5
M{120	52.2	58.5	89.5	83.7	108.7	64.0
150	37.0	49.6	90.0	101.2	106.8	72.2
180	13.0	49.0	69.0	85.0	42.1	98.9

.V., 0.3 *M*, 1 ml./kg./min.
Dog number.
njection immediately after 30 min. sample.

Table 9

Effect of THAM Administration* on Glucose Clearance/Inulin Clearance Ra of Six Phloridzinized Dogs

C_G/C_F Ratio

Time (min.)	11†	6	10	8	7	13
15	0	0	0	0	0	0
30	0	0	0	0	0	0
Phloridzin‡						
60	1.00	0.71	0.21	0.53	0.66	0.13
90	—	0.84	0.31	0.74	0.92	0.24
THAM 120	0.30	0.64	0.25	0.74	0.68	0.61
THAM 150	0.60	0.83	0.11	0.21	0.72	0.44
180	0.14	1.00	0.28	0.33	0.61	0.20

* I.V., 0.3 *M*, 1 ml./kg./min.
† Dog number.
‡ Injection immediately after 30 min. sample.

Table 10

Effect of THAM Administration* on *p*H of Blood and Urine of Six Phloridzinized Dogs

Time (min.)	11†	13	10	7	Average
			Blood *p*H		
15	7.30	7.32	7.32	7.38	7.33
30	7.31	7.35	7.31	7.39	7.34
Phloridzin‡					
60	7.27	7.33	7.26	7.36	7.31
90	7.30	7.35	7.28	7.37	7.32
THAM 120	7.64	7.56	7.59	7.60	7.60
THAM 150	7.73	7.63	7.77	7.58	7.68
180	7.57	7.52	7.52	7.46	7.52
			Urine *p*H		
15	7.58	7.26	7.67		7.50
30	7.55	7.33	7.45		7.44
Phloridzin‡					
60	7.66	7.15	7.41		7.41
90	7.39	7.10	7.30		7.26
THAM 120	7.70	7.46	7.68		7.61
THAM 150	7.88	7.66	7.88		7.81
180	7.82	7.62	7.86		7.77

* I.V., 0.3 *M*, 1 ml./kg./min.
† Dog number.
‡ Injection immediately after 30 min. sample.

urine *p*H following onset of the administration of THAM. Blood *p*H hed a maximum level of 7.68; urine *p*H, of 7.81. Thirty min. after dis- inuation of buffer, HCO_3^- increased to 35.3 mM/l. in blood and, in the e, to 70.5 mM/l.

IGURE 4 represents an attempt to correlate glucose concentrations in urine plasma with urine volume. After phloridzin, while urine glucose concen- ion increased from 0 to 1,500 mg. per cent, blood glucose showed little change urine volume increased slightly. As soon as THAM was started, urine entration fell to 250 mg. per cent and the blood concentration to 75 mg. cent. The urine volume increased from 4 to 20 ml./min.

Discussion

he marked fall of glucosuria could be due to a decrease in glucose filtered by glomeruli, or to an increase in tubular reabsorption or to dilution. In up 1 the drop in glucose clearance (C_G) is accompanied by a parallel fall in in clearance (C_{In}) indicating a decrease in glomerular filtration rate (GFR). amount of glucose filtered at the glomeruli was determined by multiplying plasma glucose (P_G) by the GFR. The results, expressed in milligrams per nute, are below the glucose "threshold" (T_G). Thus all the filtered glucose be reabsorbed normally, without the need of any increased reabsorption. he drop in inulin, glucose, and osmolar U/P ratios in both sets of experi- nts, and the negative clearance of solute-free water indicate that THAM duces an osmotic diuresis. The results obtained in the phloridzinized ani- s deserve special attention. Shapiro[14] and Lotspeich[15,16] have shown that oridzin inhibits the oxidative reaction of the citric acid cycle and the gen- ion of ATP; both also found evidence of the inhibition of phosphatases. rsh *et al.*[17] showed the inhibitory effects of phloridzin on phosphatases in eriments *in vivo* and *in vitro*. They found that the inhibitory effect was ter on the alkaline enzyme (91 per cent) than on the acid (40 per cent). y also postulated that this effect on phosphorylation was related to a de- sed oxygen consumption. Rogers,[18] studying glucose uptake in the rat hragm, concluded that it will be inhibited by acidosis. He found marked eases in the glucose uptake with insulin kept at a constant level of 0.008 /ml.; this increase was proportional to the *p*H only up to 7.4.

we assume that the inhibition of phosphatase is one of the important hanisms of the action of phloridzin, it is conceivable that THAM acts as an gonist to phloridzin by its properties of intracellular H^+ acceptor. It has n established that THAM distributes widely throughout the body water[10] a restoration of a normal *p*H in the tubular cell could interfere with the on of phloridzin.

lso, the "washout" due to the osmotic diuresis should be considered as a or which could reduce the inhibitory action of phloridzin. This has been wn to occur in *in vitro* studies on glucose transport across the intestinal osa.[19]

weakening of the action of the drug after one hour, although unlikely, ht contribute to the increased ability of the tubule to reabsorb glucose in the second hour after its administration. However, in the original observatic glucosuria was present for at least 12 hours.[4a]

All these considerations apply to the phloridzinized dogs that showed : increased tubular activity at the time of THAM administration.

In the phloridzinized dogs with an unchanged T_G after THAM, the reduc glucosuria can be accounted for by the decreased plasma glucose level and t accompanying reduction of the filtered glucose (P_GC_F) at the glomeruli. addition, the increased volume of urine diluted the unreabsorbed urinary gl cose.

SUMMARY

The effectiveness of Tris(hydroxymethyl)aminomethane (THAM) in i creasing salicyluria after intravenous injection of sodium salicylate was teste in experiments on four dogs. A marked drop of salicylate in plasma and i crease in its excretion in urine was demonstrated. Possible application of th property of THAM in the treatment of salicylate poisoning in man is discusse

The mechanism of action of THAM in reducing the glucosuria in pa createctomized or phloridzinized animals was studied in nine dogs. It w found in the pancreatectomized animals (Group 1) that the filtered gluco (P_GC_F) at the glomeruli had decreased below the "threshold" and therefore w being reabsorbed as normally. In three phloridzinized animals with unchang T_G the reduced glucosuria could be explained on the basis of a reduced filter glucose load, as in the animals of Group 1. In three other phloridzinized an mals there was an increase in the tubular reabsorption of glucose after THA administration. It was postulated that an intracellular *p*H change of t tubular cells could account for this finding.

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Discussion

S. Israels (*Department of Clinical Investigation and Research, Children's spital, Winnipeg, Man., Canada*): My colleagues, Davies, Lertzman, and th Gourley, have been interested in the effect of THAM on salicylate intoxi- ion. Early experiments with rabbits indicated that THAM could save the

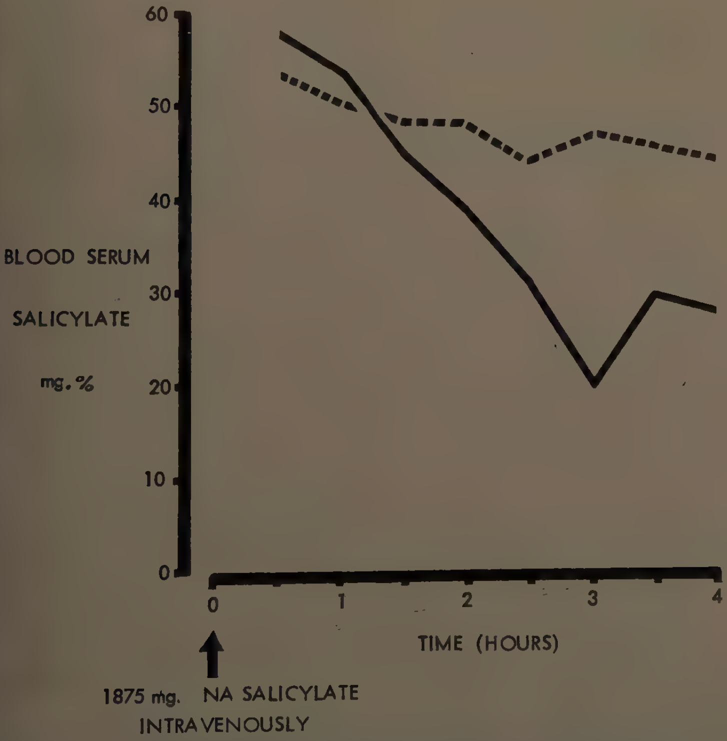

Figure 1. Treatment of salicylate intoxication (same dog 1 week apart; weight of dog, g.) Key: solid line, (240 cc. THAM) 4-hour urine excretion of salicylate 1200 mg.; broken , (240 cc. Ionosol) 4-hour urine excretion of salicylate 200 mg.

animals experimentally intoxicated by the intravenous route, but the techni difficulties of sample collections prompted us to turn to dogs for experimen work.

The dogs were intoxicated with sodium salicylate by the intravenous rou FIGURE 1 shows the results in a dog treated with THAM and the same anin treated with a multiple hypotonic electrolyte solution (Ionosol B). T THAM-treated animal excreted 64 per cent of the administered dose in 4 hou

TABLE 1

RESULTS OF EXPERIMENTAL INTOXICATION OF 7-KILOGRAM DOG WITH 2500 MILLIGRAM SODIUM SALICYLATE PER ORA

Time (hr.) from 0	Blood salicylate (mg.)	Blood pH	Blood pCO_2	Standard bicarbonate* (mEq./l.)	Urine pH	Urine bicarbonate (mEq./l.)	Salicyla excrete (mg.)
3	40	7.28	45	19	6	28	—
			THAM				
5	33	7.47	26	20	7	117	—
8	30	7.51	30	25	8	—	830
24	16	7.35	—	—	—	—	—

* Data taken from Astrup *et al.*[2]

TABLE 2

RESULTS OF THAM TREATMENT FOR SALICISM OF FOUR CHILDREN

Before THAM							Three hours after THAM					
Age (years)	Wt. (lb.)	Blood salicy-late (mg.)	Blood pCO_2	Blood pH	Std. bicarb.*	Urine pH	Blood salicy-late (mg.)	Blood pCO_2	Blood pH	Std. bicarb.	Urine pH	Sali la excr (m
2	23	37	27	7.43	18	6.0	21	31	7.47	23	8.0	4
2	28	59	26	7.43	18	5.4	24	—	—	—	7.6	5
8	55	46	—	—	—	7.0	30	—	—	—	8.0	8
16	140	54	43	7.38	25	6.5	22	41	7.47	28	7.7	18

* Data taken from Astrup *et al.*[2]

and the control excreted 11 per cent of the administered dose in the same ti and with the same volume infused. This figure of 64 per cent is far in excess the 19.3 per cent removed by Etteldorf[1] in experiments using peritoneal lava with albumen as the means of treatment.

In a 7-kg. dog experimentally intoxicated with 2500 mg. of sodium salicyl by mouth the data presented in TABLE 1 was obtained.

Four children had been treated with THAM for salicylism. The results presented in TABLE 2.

The dosage of THAM in all this work was 500 mg./kg. given as a 0.3 solution in 1 hour. No untoward effect was noted in animals or humans exc marked venospasm of the small vessels in the smaller children. We are at sent comparing the effect of intravenous sodium bicarbonate and THAM xperimental salicylism in the dog.

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THE USE OF 2-AMINO-2-HYDROXYMETHYL-1,3-PROPANEDIOL THE CORRECTION OF ADDITION ACIDOSIS AND ITS EFFECT ON SYMPATHOADRENAL ACTIVITY*

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Introduction

Massive and rapid replacement of blood with ACD (acid-citrate-dextro bank blood during the course of certain surgical procedures, or during the change transfusion of the newborn, has been accompanied by cardiovascu collapse and cardiac arrest.[1-3] The cause or causes of this type of ac cardiovascular failure are not known. The following factors have been s gested: citrate toxicity,[4,5] the high potassium content,[6] or the low hematoc of blood collected in ACD medium.

However little attention has been placed upon the possible effect of acidity of stored ACD blood in the development of cardiovascular failure duri rapid and massive transfusion. Indeed, it is noteworthy that the average of human blood, collected in ACD, ranges from 6.80 immediately after c lection to 6.20 after 3 weeks of storage. The average pH of 18 samples of ba blood collected at random during transfusions performed in the course of a we at Presbyterian Hospital, New York, N. Y., as shown in TABLE 1, was 6 (range 6.22 to 6.69).

It was, therefore, conceivable that rapid and massive transfusion of blood such an acid pH could be accompanied by an acid load that would not readily eliminated by or tolerated in the body. Indeed, a number of inve gators have suggested that a significant decrease in body pH depresses c siderably the cardiovascular response to the catecholamines.[8,9] Furthermo in preliminary experiments it has been observed that an acute massive hem rhage in dogs, followed by rapid replacement with blood collected in rout fashion in standard ACD medium, resulted in cardiac arrest and death.[10] purpose of the present series of experiments was to determine further the and mechanism of the increase in H^+ ion concentration on the genesis of car vascular failure. In addition the effects of buffers capable of counteracting acidity of transfused blood were also investigated. The two buffers used w sodium carbonate and an amine buffer, 2-amino-2-hydroxymethyl-1,3-p panediol, or Tris(hydroxymethyl)aminomethane (THAM).[11] Studies w also made to assess the effect of the addition of THAM on the composition blood collected in ACD medium.

Method

Massive transfusion studies. Four series of experiments were performed healthy mongrel dogs. The initial experimental protocol was the same in

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es. The animals were anesthetized by intravenous administration of 25 /kg. body weight of sodium pentobarbital and then bled from the femoral ry for 7 to 12 min., until they had lost an average of 45 ml. of blood per kg. ody weight. In the first series, 16 dogs were reinfused within 10 min. with ologous ACD bank blood (*p*H 6.30 to 6.90) or with autologous heparinized d acidified by the addition of 10 to 12 mEq. of 0.15 *M* HCl/500 ml. blood. he second series, 16 other dogs were treated similarly except that the transd blood was titrated with 12 to 15/m*M* of 0.3 or 0.6 *M* solution of THAM, hat the blood *p*H was restored to 7.30 to 7.90. Arterial blood samples were en before, during, and following hemorrhage and during transfusion for pH, $_2$, K^+, Na^+, Cl^-, and $p\text{CO}_2$ analyses. The *p*H was measured with a microillary glass electrode thermostated at 37° C. and a Knick model 35 *p*H meter. microgasometer of Natelson[12] was employed for determination of plasma $_2$ content. HCO_3^- and $p\text{CO}_2$ were calculated by the Henderson-Hasselh equation. Chloride was measured with the Cotlove Chloridometer,[13] concentrations of sodium and potassium with the Patwin flame photometer. od pressure and electrocardiogram were continuously monitored with a ct-writing recorder. In several experiments of both series, plasma cate-

TABLE 1

*p*H OF ACID-CITRATE-DEXTROSE (ACD) BANK BLOOD AT TIME OF ADMINISTRATION

No. of samples	Age (days)	Average	Range
18	5–16	6.49	6.22–6.69

lamine concentrations were determined by a modification of the method of l-Malherbe and Bone.[14] This modification was identical to that employed Manger[15] except that, in the present study, two interchangeable secondary rs were used to permit the quantification of epinephrine and norepinephrine. peak of the wave length transmitted by one of these filters (Corning filter 4, forming color specification 2-73) is between 578 and 588 mμ. The peak he wave length transmitted by the other filter (a combination of Corning r 5562 and 3385, forming respectively color specification 5-61 and 3-71) is roximately 490 mμ. Results for the catecholamines were accurate to within 0 per cent.

n a third series, 8 dogs were treated similarly, except that the transfused d was treated with 7 to 10.5 m*M* of 0.15 *M* sodium carbonate. In a th series, 7 dogs were simultaneously given, during transfusion of acid d, 14 to 18 mEq. of calcium chloride administered intravenously.

ffects of THAM on composition of human blood. Two series of experiments conducted on human blood collected in standard ACD solution, N. I. H. ula B. In the first study, blood from the same subject was collected into 250-ml. bottles containing 60 ml. of ACD solution. Following withdrawal e blood, 15 to 30 m*M* of THAM was added to one of the aliquots. Samples taken from both bottles immediately after collection, and at regular inals for a period of 4 weeks, for measurement of platelet count, prothrombin time, plasma electrolytes, pH, CO_2, pCO_2, O_2 saturation, and ammonia leve In the second series, the effect of THAM added to ACD blood just befo transfusion was studied.* Survival time of reinjected autologous red blood ce was determined in a group of 6 volunteers. The red cells were labeled wi radioactive chromium by the addition, to 35 to 40 ml. of ACD blood, $Na_2Cr^{51}O_4$ of high specific activity, a method described by Ultmann.[16] T mixture was gently agitated for about 10 min. After 1 hour, 150 mg. of ascorb acid was added. The cells, with the plasma and ascorbic acid, were admin tered to a control subject. The other volunteer received a mixture of the cel plasma, and ascorbic acid to which THAM had been added in amount sufficie to buffer the blood to a pH of 7.40. Twenty-four hours later, and at approp ate intervals thereafter over a 30-day period (usually every 2 or 3 days), sampl

TABLE 2

TRANSFUSION ACD BLOOD

Hemorrhage			ACD Blood					Dog arterial pH			Fat
Total (ml.)	ml./kg.	Time (min.)	Vol. (ml.)	Time (min.)	Age (days)	Type	pH	Control	Post-bleeding	Post-transfusion	
425	47.2	10	500	8	7	Homo	6.38	7.36	7.38	6.95	D*
500	50.0	10	510	7	10	Homo	6.40	7.50	7.19	6.94	D
500	56.0	10	520	7	10	Homo	6.32	7.41	7.38	6.95	D
500	47.5	12	530	8	10	Homo	6.34	7.38	7.41	6.80	D
425	50.5	10	475	9	4	Homo	6.45	7.36	7.36	6.98	D
350	58.0	10	400	8	2	Homo	6.45	7.30	7.31	6.80	D
375	41.6	10	420	10	2	Homo	6.55	7.31	7.24	6.84	D
400	44.0	11	450	8	0	Auto	6.91	7.28	7.36	7.13	S†
500	44.0	12	500	12	0	Auto	6.51	7.33	7.48	6.88	D
450	44.0	10	480	10	7	Homo	6.69	7.32	7.47	6.87	D
Av 443	48.3	10.5	479	8.7	5.2		6.50	7.36	7.36	6.91	9/10

* Died.
† Survived.

of venous blood were drawn for determination of hematocrit and red cell C activity. The radioactivity of the blood was determined by counting 3- aliquots of packed red cells in a well-type scintillation counter. Counts we corrected for background. No correction was made for elution of Cr^{51} fro the red cells. The data thus obtained were expressed as percentages of t radioactivity at 24 hours. The normal half life of red blood cells tagged by t method is 28 to 40 days. Red blood cell survival was studied under su conditions after 1, 2, and 3 weeks of storage in ACD blood; THAM was add just before the tagging and the reinjection of the cells into 3 of the test subjec

Results

Massive transfusion studies. The results of series 1 and 2 are shown TABLES 2 to 7. Thirteen of 16 animals treated by rapid administration of a od presented cardiac arrest during transfusion. The average pH at the time leath was below 7.00, and the $p\text{CO}_2$ above 45 mm.Hg (FIGURE 1). These mals had an increase in plasma Na^+, a decrease in Cl^- and HCO_3^-, but no nge in K^+ (FIGURE 2). The mean concentration of plasma catecholamines

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* Lyophilized preparation kindly provided by Abbott Laboratories, North Chicago, Il

TABLE 3

TRANSFUSION ACD BLOOD + THAM

Hemorrhage			ACD Blood + THAM							Dog arterial pH			Fate
otal nl.)	ml./ kg.	Time (min.)	Vol. (ml.)	Time (min.)	Age (days)	pH (unbuf.)	pH (buf.)	Type	m M 0.3 to 0.6 M THAM	Control	Post-bleeding	Post-transfusion	
500	50.0	8	520	12	10	6.73	7.55	Homo	15	7.33	7.36	7.30	S*
450	46.0	10	480	11	10	6.60	7.80	—	15	7.37	7.36	7.32	S
450	50.5	10	480	13	2	6.80	7.70	—	15	7.39	7.30	7.12	S
600	46.2	6	600	10	5	6.70	7.40	—	12	7.31	7.29	7.03	S
700	50.0	15	700	9	1	6.44	7.67	—	14	7.44	7.30	7.15	D†
450	50.0	12	470	10	2	6.54	7.40	—	13	7.42	7.31	7.18	S
470	57.0	10	480	12	2	6.78	7.51	—	14	7.26	7.29	7.20	S
520	47.0	12	530	13	2	6.82	7.53	—	14	7.35	7.30	7.29	S
500	43.9	10	510	10	2	6.80	7.44	—	12	7.38	7.43	7.44	S
520	49.5	7	540	7	1	6.70	7.57	—	13	7.37	7.42	7.40	D
516	49.0	10	531	11	4	6.69	7.56		14	7.36	7.34	7.24	8/10 S

Survived.
Died.

TABLE 4

TRANSFUSION AUTOLOGOUS BLOOD + HCl

Hemorrhage			Auto blood + HCl				Dog arterial pH			Fate
ol. ni.)	ml./kg.	Time (min.)	Vol. (ml.)	Time (min.)	0.15 M HCl (mEq.)	pH	Control	Post-bleeding	Post-transfusion	
500	42.4	8	575	9	15	6.43	7.38	7.44	6.84	D*
600	41.4	9	650	10	17	6.51	7.42	7.48	6.91	D
300	44.1	8	335	8	9	6.61	7.43	7.54	7.00	D
330	52.2	6	365	7	10	6.31	7.54	7.52	7.03	D
600	46.0	8	550	8	14	6.47	7.37	7.38	6.94	D
750	53.5	9	750	10	18	6.48	7.35	7.43	7.05	S†
513	46.6	8	538	8.7	13.8	6.47	7.42	7.46	6.96	5/6 D

Died.
Survived.

significantly increased, particularly in animals with the greatest degree of losis (TABLE 6).

Vhen transfused blood was titrated with THAM to an average pH of 7.54, of 16 animals survived the procedure. Arterial blood pH still decreased owing onset of transfusion, indicating that the restored cardiac function was using areas where acid metabolism had accumulated. However arterial od pH did not fall below 7.00 in the course of reinfusion, and $p\text{CO}_2$ remained below 40 mm. Hg (FIGURE 1). The plasma K^+, Na^+, and Cl^- did not chan significantly, whereas a slight increase in HCO_3^- occurred (FIGURE 2). At tl end of transfusion the mean concentrations of plasma catecholamines we within the normal range (TABLE 7).

Administration of acid blood to 2 dogs (upper tracings of FIGURES 3 and resulted in a decrease of *p*H to 7.00 and 6.84, a transient elevation of bloc

TABLE 5
TRANSFUSION AUTOLOGOUS BLOOD + HCl AND THAM

Hemorrhage			Auto blood + HCl + THAM					Dog arterial *p*H			Fate
Vol. (ml.)	ml./kg.	Time (min.)	Vol. (ml.)	Time (min.)	0.15 *M* HCl (mEq.)	0.3 *M* THAM (mM)	*p*H	Control	Post-bleeding	Post-transfusion	
420	44.2	9	470	11	12	13	7.33	7.38	7.35	7.19	S*
400	48.7	9	500	8	18	33	7.44	7.41	7.59	7.23	S
500	44.2	8	500	10	18	30	7.86	7.38	7.27	7.22	S
400	49.0	9	450	12	19.5	24	7.53	7.33	7.51	7.19	S
420	45.0	9	480	10	13	15	7.46	7.30	7.36	7.23	S
450	47.5	8	520	10	15	14	7.47	7.35	7.49	7.29	S
Av 432	46.4	8.7	487	10	16	21.5	7.52	7.36	7.43	7.22	6/6

* Survived.

TABLE 6
THE *p*H AND PLASMA CATECHOLAMINES CONCENTRATION IN 6 DOGS DURING RAPID HEMORRHAGE REPLACED WITH ACID BLOOD

	Donor blood	Control	After hemorrhage		After transfusion of acid blood	
			250–300	400–500	+250	+500
Av *p*H	6.54	7.33	7.34	7.34	7.03	6.99
Av Epi*	5.5	0.8	6.7	19.6	15.3	19.3
Av NorE†	3.3	3.1	1.8	3.2	5.4	4.9

* Epinephrine. μg./l.
† Norepinephrine. μg./l.

TABLE 7
THE *p*H AND PLASMA CATECHOLAMINES CONCENTRATION IN 5 DOGS DURING RAPID HEMORRHAGE REPLACED WITH BLOOD AND THAM

	Donor blood	Control	After hemorrhage		After transfusion + THAM	
			250–300	400–500	+250	+500
Av *p*H	7.40	7.33	7.27	7.35	7.23	7.21
Av Epi*	5.1	0.0	8.1	10.7	9.4	0.4
Av NorE†	3.4	6.0	3.9	2.5	2.4	3.5

* Epinephrine. μg./l.
† Norepinephrine. μg/l.

ssure with subsequent hypotension, and death of the animals. However
ninistration of buffered blood to the other 2 dogs (lower tracings of FIG-
ES 3 and 4) resulted in the maintenance of a pH above 7.17, a return of the
od pressure toward normal, and survival of the animals.

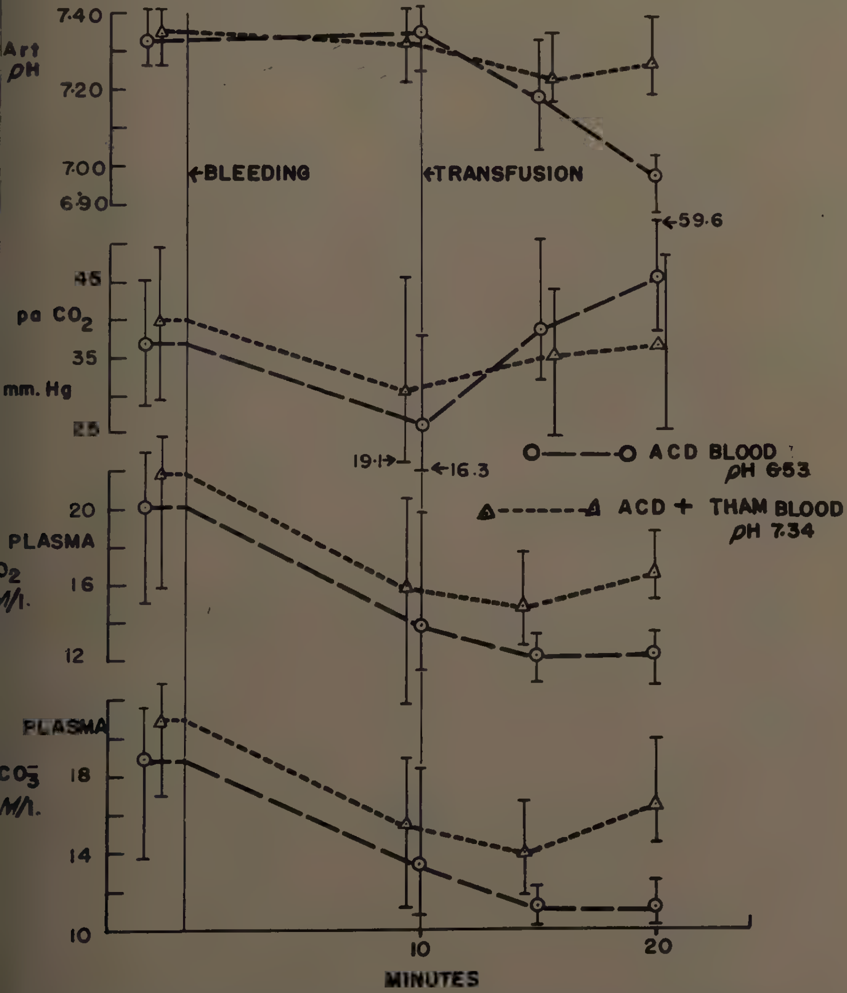


FIGURE 1. Effect of rapid massive hemorrhage, followed by replacement with ACD
od or ACD blood buffered with THAM on arterial pH, pCO$_2$, and plasma CO$_2$ and
O_3^- content of 5 dogs.

The results of the third series of experiments indicate that it is possible to
rate ACD blood back to a normal pH with sodium carbonate (TABLE 8). As
y be seen, the average pH after transfusion was 7.26, and 7 of 8 dogs sur-
ved. In this series, signs of marked tetany were frequently observed even
ough the animals were anesthetized.

TABLE 9 shows the results of the last series and indicates that infusion calcium chloride during transfusion of acid blood was followed by survival of of 7 dogs.

Effects of THAM on the composition of human blood. THAM and sodiu carbonate are two buffers that can be used to titrate standard ACD solution to

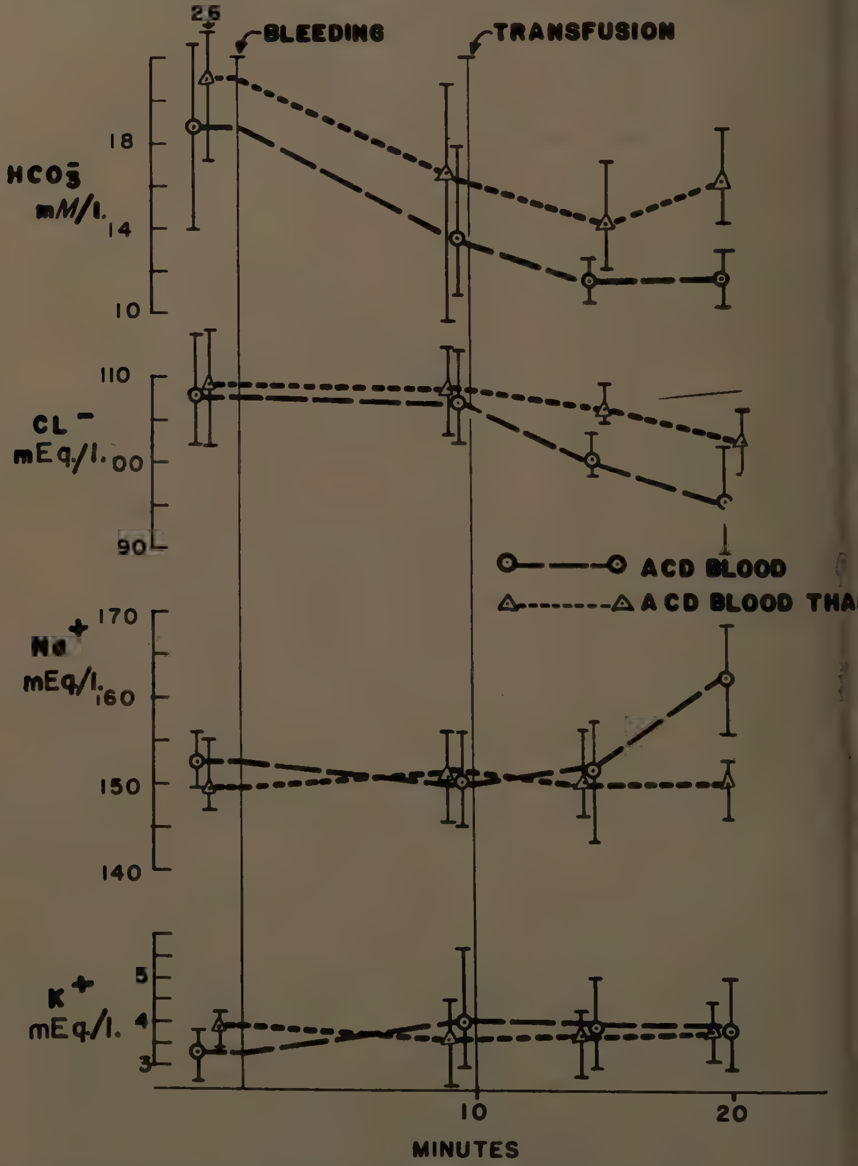
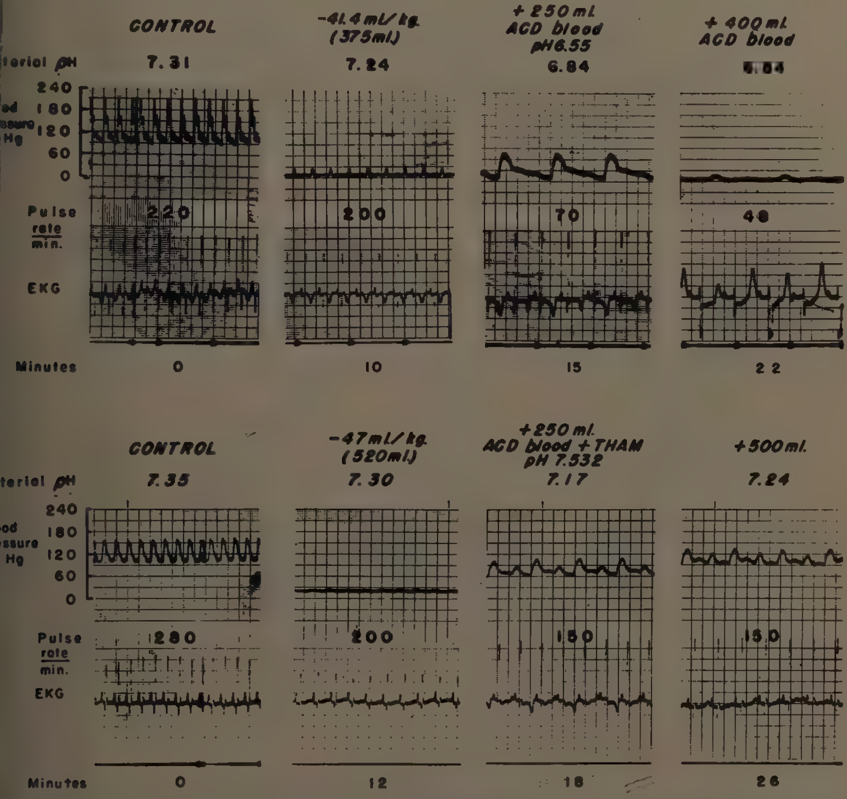


FIGURE 2. Effect of rapid massive hemorrhage, followed by replacement with ACD bl or ACD blood buffered with THAM on HCO_3^-, Cl^-, Na^+, and K^+ concentration in arte blood of 5 dogs.

mal *p*H (FIGURE 5). Standard ACD solution (N.I.H. formula B, which is f as concentrated as formula A) has a *p*H of 4.85 (*p*H measured at body nperature); 120 ml. of this solution, which is used to preserve 500 ml. of blood, ntains 10 mEq. of titratable acid. Immediately after collection, therefore, *p*H of ACD blood is in the vicinity of 6.80. Glycolysis, which takes place ing storage, will bring the *p*H to 6.40 or lower after 3 weeks.



IGURE 3. Effect of rapid massive hemorrhage, followed by replacement with ACD blood *per tracing*) or ACD blood buffered with THAM (*lower tracing*).

he results of an *in vitro* study are shown in FIGURES 6 to 9. The average ial platelet count in 3 samples of ACD blood was 160,000, whereas that of od samples from the same 3 donors collected with ACD plus THAM was roximately 96,000 (FIGURE 6). The platelet counts decreased progressively oth the ACD blood and in the ACD-plus-THAM blood during the first 3 ks following collection. However in all samples the platelets were lower in blood containing THAM. The reason for this discrepancy is uncertain. ing a 4-week period of observation there was no significant difference be- en the prothrombin time of ACD blood and ACD blood that had been buff- l with THAM (FIGURE 7). The addition of THAM to ACD blood results greater concentration of HCO_3^-, a lower pCO_2, and a higher *p*H during

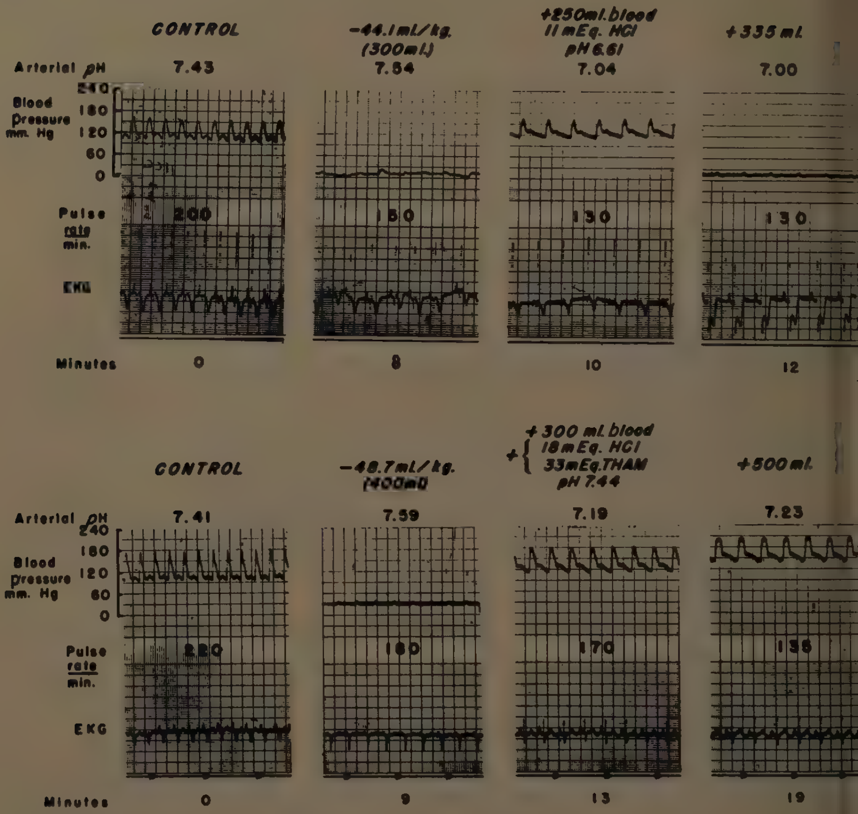


FIGURE 4. Effect of rapid massive hemorrhage, followed by replacement with acid blo (*upper tracing*) or acid blood buffered with THAM (*lower tracing*).

TABLE 8
TRANSFUSION OF ACD BLOOD + 0.15 M Na_2CO_3

Hemorrhage			Transfusion							Dog arterial pH			
Vol. (ml.)	ml./kg.	Time (min.)	Vol. (ml.)	Time (min.)	Age (days)	pH (unbuf.)	pH (buf.)	Type	Na_2CO_3 (mM)	Control	Post-bleeding	Post-transfusion	Fat
465	42	15	500	18	5	6.72	7.42	Homo	7.0	7.42	7.22	7.08	S*
400	40	16	510	15	0	6.64	7.22	Auto	7.0	7.42	7.37	7.39	S
330	49	14	400	13	3	6.61	7.31	Homo	7.0	7.32	7.38	7.24	S
490	43	12	560	15	0	6.80	7.37	Auto	9.5	7.39	7.23	7.25	S
460	37	19	550	10	6	6.59	7.30	Homo	6.0	7.29	7.43	7.26	D
500	49	11	580	7	0	6.37	7.31	Auto	7.5	7.31	7.36	7.32	S
700	47	15	760	20	5	6.85	7.29	Homo	7.5	7.46	7.42	7.30	S
600	44	10	680	18	0	6.72	7.30	Auto	10.5	7.37	7.45	7.22	S
Av 493	44	14	568	14.5	2.4	6.66	7.315		7.75	7.37	7.36	7.26	7/8

* Survived.
† Died.

TABLE 9

TRANSFUSION OF ACD BLOOD + SIMULTANEOUS INFUSION OF $CaCl_2$

Hemorrhage			Transfusion			Titratable acid added		Blood pH	$CaCl_2$ admin. (mEq.)	Arterial pH			Fate
al .)	ml./kg.	Time (min.)	Vol. (ml.)	Time (min.)	Type	Acid	mEq.			Control	Post-bleeding	Post-transfusion	
00	48	15	680	9	Homo	ACD	13	6.84	18	7.47	7.52	7.26	S*
00	48	15	690	13	Homo	ACD	11	6.96	14	7.36	7.51	6.87	D†
40	50	12	600	12	Homo	ACD	10	6.67	18	7.39	7.46	7.01	S
30	50	12	700	14	Homo	HCl	12	6.68	18	7.38	7.37	7.05	S
10	51	14	560	10	Homo	HCl	10	6.40	14	7.35	7.28	7.12	S
80	50	10	580	11	Homo	HCl	9	6.60	16	7.35	7.28	7.07	S
40	47	17	510	12	Homo	HCl	7.5	6.70	14	7.29	7.42	7.18	S
57	49.1	13.6	617	11.6			10.4	6.69	16	7.37	7.41	7.08	6/7 S

Survived.
Died.

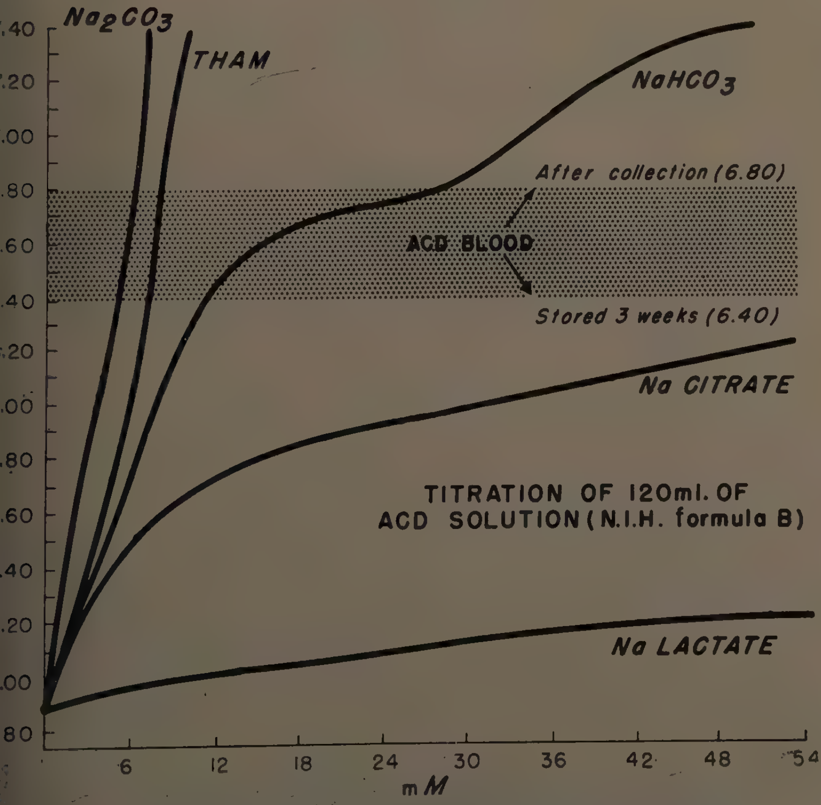


FIGURE 5. Effect of titration of standard ACD solution with various buffers.

27 days of observation (FIGURE 8). The CO_2 content was initially greate the ACD blood to which THAM had been added but was less than ACD bl during the last week of observation. ACD blood buffered with THAM h significantly lower concentration of plasma Na^+, whereas no significant ference was noted in concentration of Cl^- or in the O_2 saturation (FIGURE However, within a week or more a very significant increase in the plasma and NH_4^+ was noted in the ACD blood to which THAM had been ad

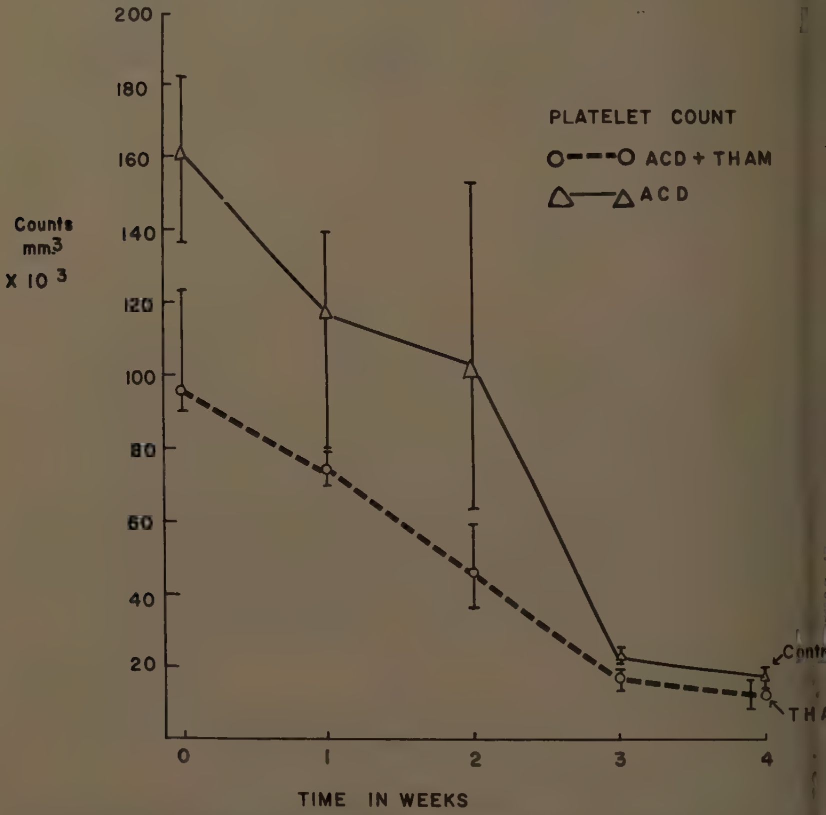


FIGURE 6. Platelet counts of human blood collected in ACD with and without THA

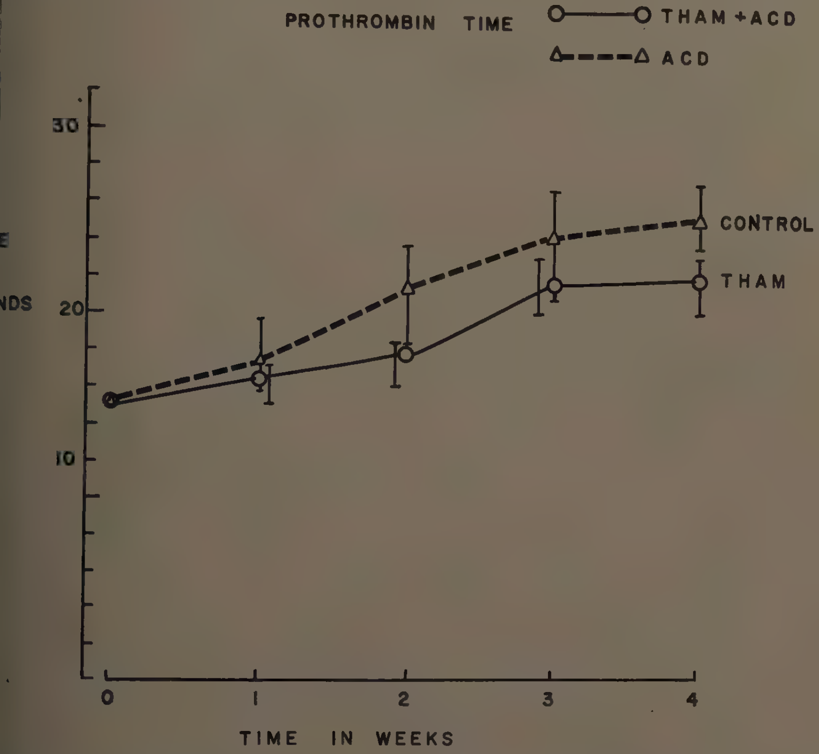
This increase was felt to be due to the destruction of red blood cells. Bec of this hypothesis, we have also studied the effect on the survival time of blood cells to which THAM was added just before transfusion.

Results of the second series of studies are shown in FIGURES 10 to 12. T was no significant difference in the survival time of erythrocytes of blood lected in ACD or in ACD to which THAM had been added prior to transfu

Discussion

The fact that death resulted with about the same frequency after transfu of blood acidified by the addition of HCl as with blood collected in ACD

uggested that the lowered pH per se was an important factor in causing
ovascular collapse. This observation would also indicate that citrate per
s little inherent toxicity, as already emphasized by Howland *et al.*[17] Fur-
nore, the survival of the dogs transfused with ACD blood buffered with
m carbonate to a normal pH indicates the importance of this factor in the
tenance of cardiovascular homeostasis under conditions of acute stress.
e results further emphasize the danger inherent in massive and rapid



URE 7. Prothrombin time of human blood collected in ACD medium and in ACD
m buffered with THAM.

usion of acid blood (whether acidity is due to the presence of ACD solu-
r acid).

e exact mechanism causing death due to transfusion acidosis is uncertain.
lectrolyte studies that were performed during the transfusion with ACD
indicate that the only disturbance is a lowering in HCO_3^- and in Cl^-, and
in Na^+ (FIGURE 2). It is also apparent that these disturbances are cor-
when the acidity of ACD blood is buffered with THAM. Noteworthy
fact that there were no significant changes in plasma K^+ levels of arterial
in treated or untreated animals. These observations do not agree with
of LeVeen *et al.*,[6] who relate cardiac arrest following massive transfusion
high potassium levels of ACD-stored blood. However, the study by

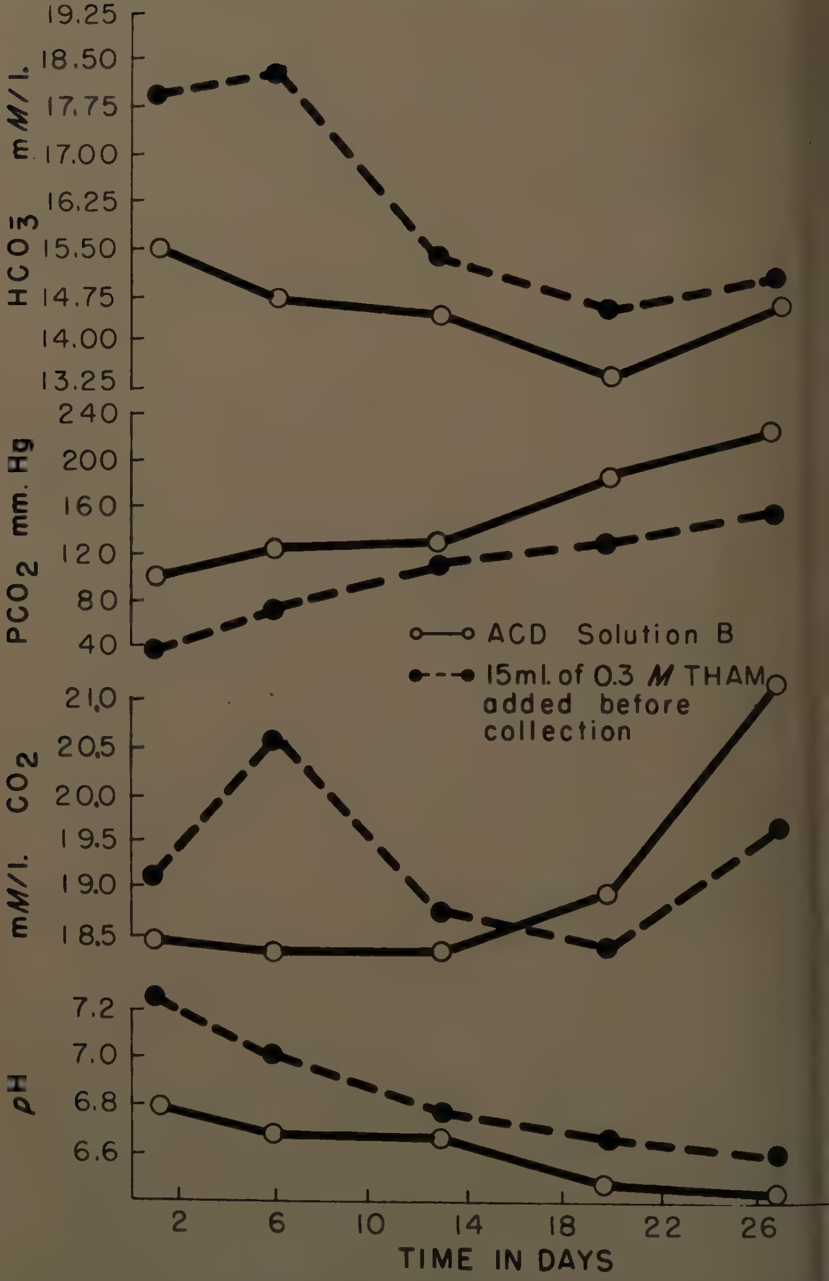
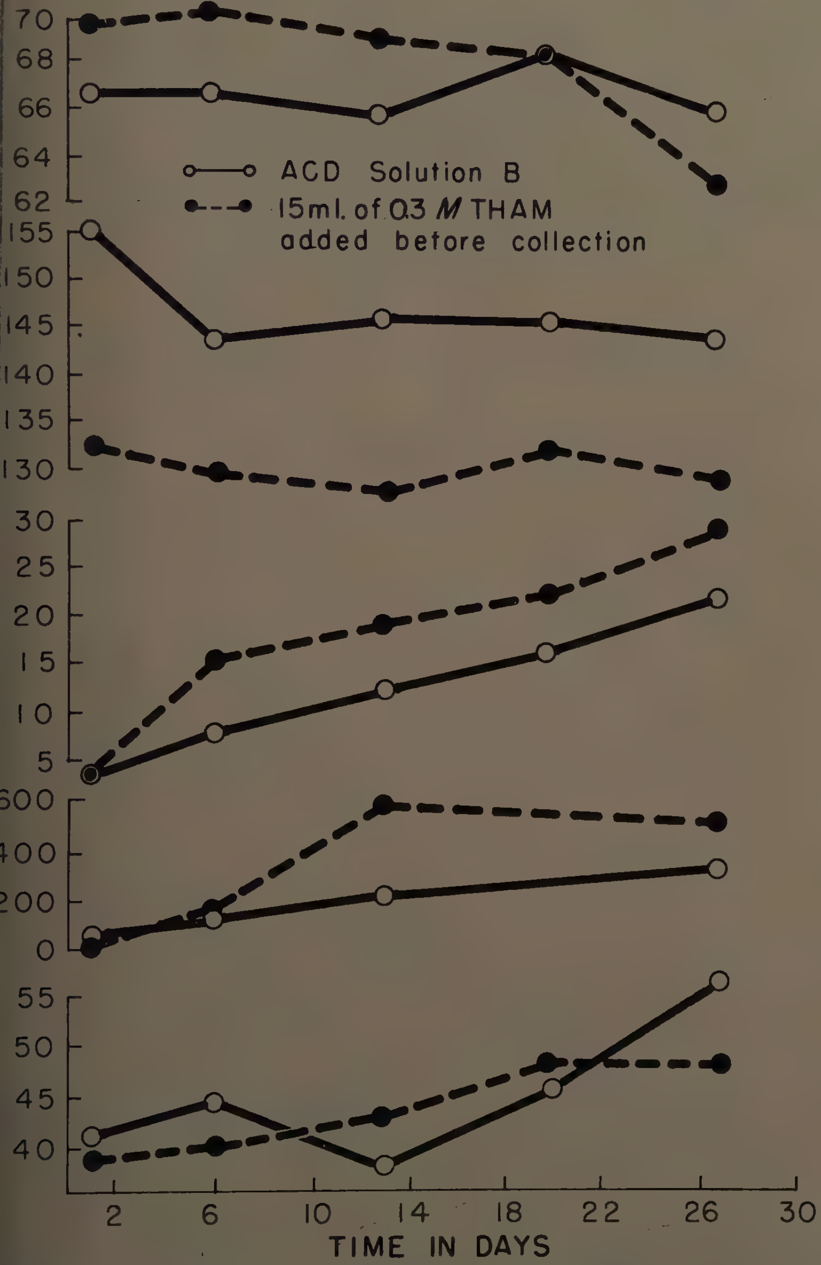


FIGURE 8. *In vitro* values of HCO_3^-, pCO_2, CO_2, and *p*H in two 250-ml. aliqu stored human ACD blood (the two aliquots are from the same donor). THAM (15 ml. added to one of the aliquots (*dotted line*).



URE 9. *In vitro* values of Cl^-, Na^+, K^+, NH_4^+, and O_2 saturation in two 250-ml. s of stored human ACD blood (the two aliquots are from the same donor). THAM) was added to one of the aliquots (*dotted line*).

LeVeen *et al.* was made on human subjects, and it is known that the dis bution of K^+ in man and in dog is quite different. It must also be pointed that the increase in Na^+, observed in animals transfused with ACD solution not a primary factor in the death of the animal since normal levels were served in those transfused with autologous blood acidified by addition of H this last group also had a very high mortality rate. Furthermore, when blood was titrated with Na_2CO_3, high sodium levels were also observed a transfusion and this group had a high survival rate. It seems that the fal

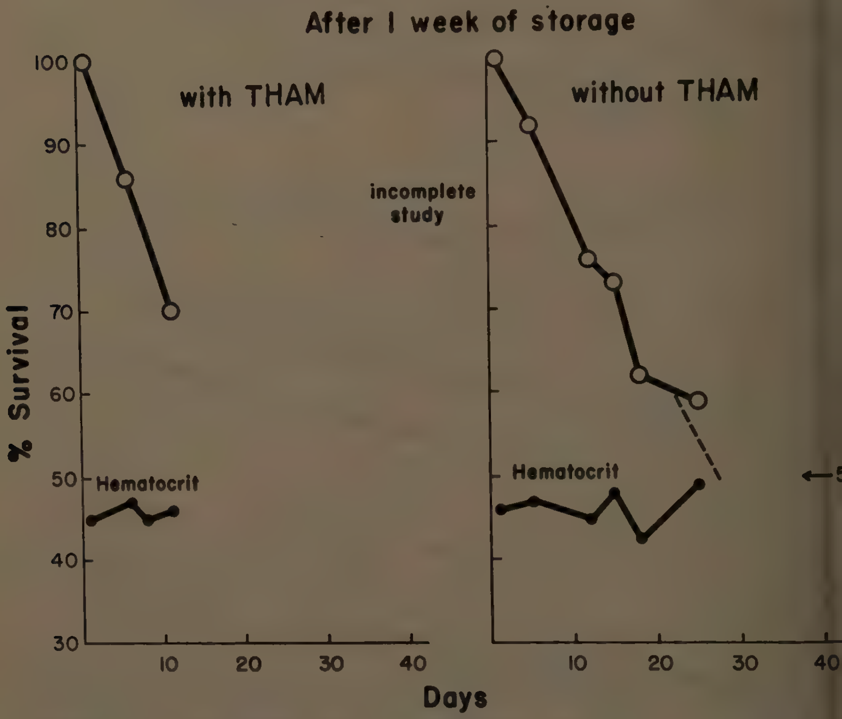
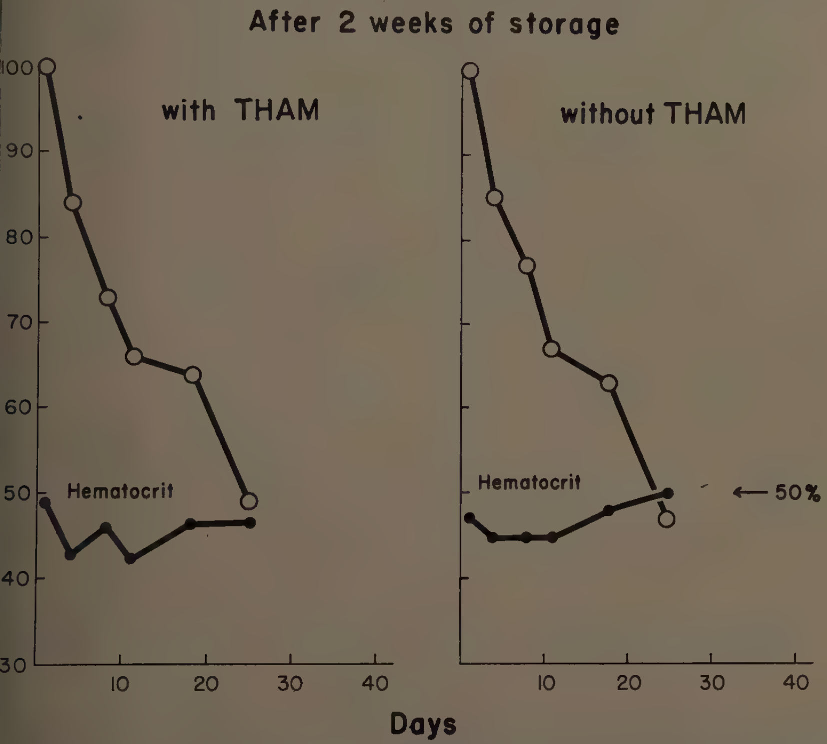


FIGURE 10. *In vivo* survival time of human erythrocytes in ACD blood and ACD b to which THAM had been added immediately prior to transfusion, following one wee storage.

HCO_3^- may be of significant importance, though it was observed in the ani receiving acid blood and a simultaneous intravenous injection of calcium c ride. Calcium chloride exerts a "cardiotonic" action[18] that results in maintenance of an adequate cardiovascular function. $CaCl_2$ may also res to normal levels ionized calcium, which is depressed during acidosis. In present series of experiments it is not possible to relate cardiovascular coll to any specific shift in K^+, Na^+, Cl^-, or HCO_3^-. The role of ionized calc should be investigated.

The role played by the sympathoadrenal system during massive hemorr is well established.[19] Both the hypotension secondary to hemorrhage an significant decrease in blood *p*H activate the sympathoadrenal system the secretion of catecholamines. However the present experiments ate that, following massive and rapid hemorrhage in the dog, the replace- of blood loss and the presence of normal or markedly increased concentra- of endogenous catecholamines will not sustain normal blood pressure and ac function if the pH of the blood is significantly low (TABLE 6). Under conditions of severe acidosis, in order to maintain adequate cardiovascular ion it is necessary to administer large amounts of epinephrine or norepi- rine during the transfusion of acid blood (0.5 to 1.0 mg./500 ml. of blood). restoration of the blood volume and pressure toward normal, and the tenance of a relatively normal blood pH with THAM, the plasma con- ation of catecholamines returns toward normal.



GURE 11. *In vivo* survival time of human erythrocytes in ACD blood and ACD blood ich THAM had been added immediately prior to transfusion, following two weeks of e.

ny investigators have found that the stress of hemorrhagic shock causes lation of the entire sympathoadrenal system, with a resulting increased ion of catecholamines[20] and corticosteroids.[21] We have also found that owering of the pH per se, which results from respiratory acidosis, will a stimulation of the sympathoadrenal system and, thereby, an increased ion of catecholamines and corticosteroids. In a previous publication[22] s reported that if THAM is administered to an animal during respiratory acidosis the increased plasma concentrations of catecholamines will return ward normal levels despite the presence of a markedly elevated pCO_2. Mit man *et al.*[23] also reported that the increased secretion of hydrocortisone (co pound F) occurring during respiratory acidosis can be prevented by maintain a relatively normal pH with the administration of THAM. Experiments 10 dogs were performed in the following manner: after denitrogenation mechanical ventilation with 100 per cent O_2, respiratory acidosis was induc in anesthetized dogs by maintaining them in apneic oxygenation for 1 ho

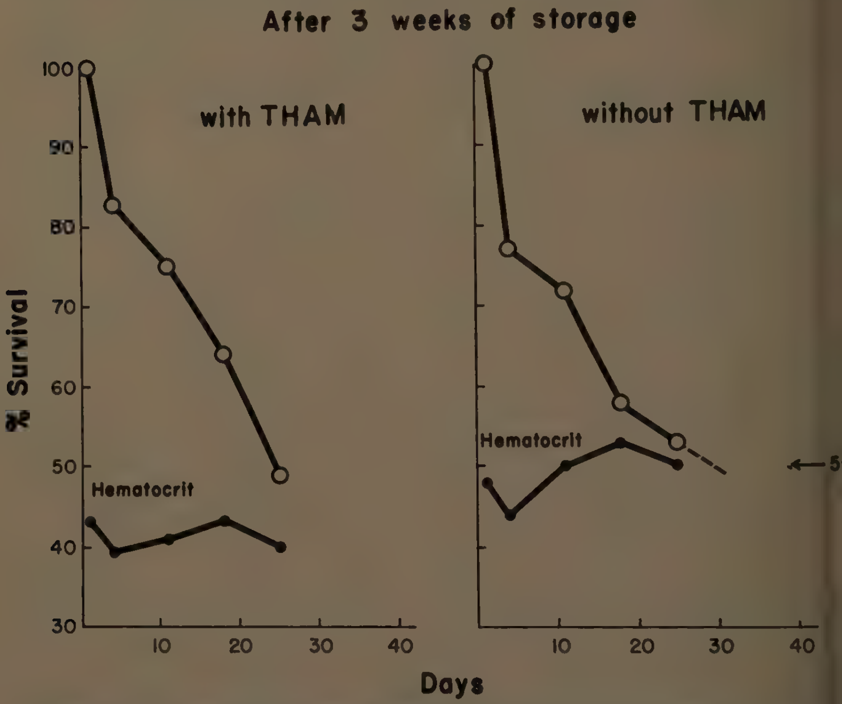


FIGURE 12. *In vivo* survival time of human erythrocytes in ACD blood and ACD b to which THAM had been added immediately prior to transfusion, following three week storage.

Systemic arterial and venous blood pHs and the adrenal vein blood pH fel 6.9 to 6.7; $PaCO_2$ rose to 180 to 300 mm.Hg; adrenal blood flow collected fro catheter inserted into the phrenicoabdominal vein was unchanged; adre vein concentration of compound F rose; but adrenal vein O_2 saturation remai normal. A recovery period on mechanical ventilation was then permit followed by another hour of apneic oxygenation during which the dogs recei intravenously 0.3 mM/kg./min. of 0.3 M THAM. The average pH remai within 0.1 pH unit of the control; adrenal blood flow rose; and the adrenal concentration of compound F, which had remained high through the recov period, fell. After a second recovery period, all values were back to con level (TABLE 10). These data suggest that the stimulation of the adrenal hat characterizes hypercapnic acidosis is also prevented by THAM. It s likely that an interrelationship between the mechanisms of the action of holamines and corticosteroids may exist during circulatory shock and us types of acidosis, and it is significant that THAM will act on both. ever, the precise interrelationship and mechanisms of action involved re- complex and uncertain. Whether THAM acts on the sympathoadrenal m through a *p*H effect per se, or through a specific action, still is to be rmined. The massive transfusion studies indicate that whenever possible fusion of ACD blood should be carried out at a slow rate (0.5 to 1.0 ml./ nin. or less), and should be accompanied by hyperventilation. The table acid contained in each pint of blood will displace 250 ml. of CO_2 h must be eliminated by the lungs in order to maintain a normal *p*H. als receiving acid blood at a slow rate (0.5 to 1.0 ml./kg./min.) and under anical ventilation did not present the cardiovascular collapse that was rved with rapid transfusion (3 to 4 ml./kg./min.). FIGURE 13 shows the

TABLE 10

EFFECT OF RESPIRATORY ACIDOSIS ON PERIPHERAL VENOUS HYDROCORTISONE LEVELS IN 10 DOGS (AVERAGE)

	pCO_2 (mm. Hg)	HCO_3^- (mEq./l.)	*p*H (Arterial)	Micrograms F/100 cc. plasma
rol	22.1	15.22	7.48	30.5
r AO*	282.0	27.26	6.61	56.6
control	25.3	15.23	7.40	54.3
r AO—THAM‡	59.6	42.45	7.38	30.0
ontrol	24.8	28.90	7.68	43.1

pneic oxygenation.
Iechanical ventilation.
THAM 700 to 1000 ml. 0.3 *M* solution.

ence of the rate of infusion of ACD blood and ACD blood buffered with M on *p*H and on pCO_2 of arterial blood ($PaCO_2$). There appears to be a ical zone" below *p*H 7.05. If the blood *p*H of the dog decreases below 7.00, ighly probable that the animal will die. Following rapid massive hemor- , transfusion with ACD blood at the rate of 3 to 4 ml./kg./min. caused H to drop to the "critical zone" and the pCO_2 to increase from 25 to about m. Hg. Such an increase in $PaCO_2$ indicates an inadequate respiratory ensation to the acid load. However, transfusion of ACD blood at the of 0.5 to 1.0 ml./kg./min., or ACD blood plus THAM at the rate of 3 to /kg./min., prevented the *p*H from falling below the "critical zone" and ted in a $PaCO_2$ increase, from 25 to about 33 mm.Hg.

e results of our *in vitro* studies on stored ACD blood buffered to a normal vith THAM further confirm that blood preservation in ACD medium is um at an acid *p*H (6.50 to 7.00) as shown by Rapaport.[24] The greater K^+ and NH_4^+ in the plasma of ACD blood buffered to a normal *p*H with M is indicative of a faster rate of breakdown of the red blood cells. How- our studies on red blood cell survival *in vivo* indicate that the addition of a solution of THAM, in an amount sufficient to restore ACD blood to a

normal pH just before transfusion, does not alter the survival of the red b
cells. About 10 to 12 mM of a 0.3 M solution (35 ml.) is required to restore
normal pH (7.30 to 7.40) the 500 ml. of blood collected in 120 ml. of ACD
tion B. This represents 1.2 gm. of THAM, and it has been established

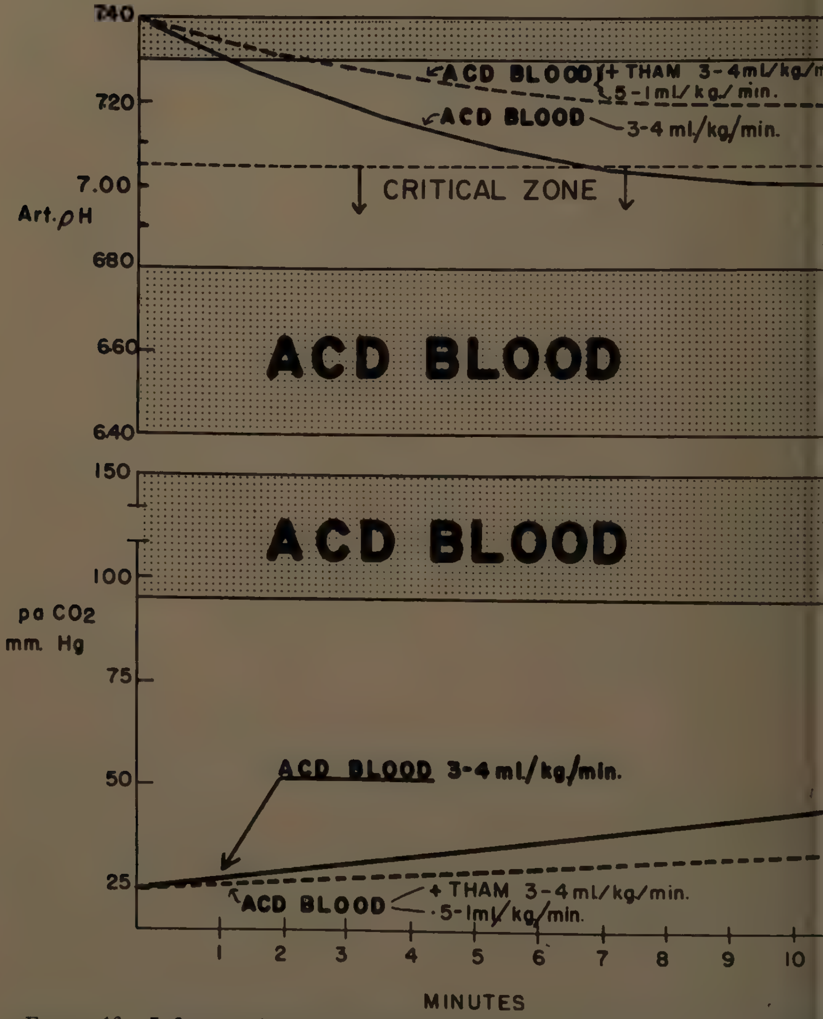


FIGURE 13. Influence of rate of infusion of ACD blood and ACD blood buffered THAM on pH and on pCO$_2$ of arterial blood (PaCO$_2$).

500 mg. of THAM per kg. of body weight can be administered safely healthy adult over a 1-hour period.[25] However, the use of THAM to b ACD blood for replacement of massive hemorrhage must await further inv gation. If utilization of THAM proves to be possible, it should not pre the necessity of developing a new medium for the preservtion of blood at a mal pH. Such attempts are presently being made.[26, 27]

Summary and Conclusions

assive and rapid hemorrhage of dogs (50 ml./kg. in 10 min.), followed by a
nsfusion of ACD blood or blood acidified by addition of HCl (pH 6.40 and
) at the rate of 3 to 4 ml./kg./min., is accompanied by cardiac arrest in 80
cent of the cases. Survival of the animals can be obtained when the
nsfused blood is buffered with THAM or $Na_2 CO_3$ (10 to 12 mEq./500 ml.
CD-preserved blood). THAM is better tolerated than equivalent amounts
Na_2CO_3. The mechanism of this cardiovascular collapse accompanying
nsfusion acidosis is still to be determined. Electrolyte shifts of K^+, Na^+,
, or HCO_3^- cannot account for it, and the stimulation of the sympatho-
enal system cannot prevent it. The addition of 10 mM of 0.3 M THAM,
before transfusion, to 500 ml. of ACD-preserved blood will restore pH to
mal and will not affect the survival of the red blood cells. Thirty times
amount has been administered to healthy subjects without untoward ef-
s. However the use of THAM to buffer stored ACD blood before ad-
nistration in clinical practice should await further investigation.

his work points out the necessity of developing a medium for the preserva-
n of blood at a normal pH that could be used with less danger in massive
nsfusions, such as those required by severe hemorrhage, extracorporeal
ulation, and exchange transfusion of the newborn. Meanwhile, whenever
sible, transfusions of ACD blood should be carried out at a rate not exceeding
l./kg./min. and with moderate hyperventilation. Furthermore, the present
dies also indicate that simultaneous intravenous administration of calcium
oride during massive and rapid transfusion has in dogs a protective effect
inst cardiovascular collapse.

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FFECTS OF 2-AMINO-2-HYDROXYMETHYL-1,3-PROPANEDIOL ON THE PULMONARY HYPERTENSION OF HYPERCAPNIC ACIDOSIS

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INTRODUCTION

ypercapnic acidosis of apneic oxygenation is accompanied by a marked ease in mean pulmonary artery pressure in the dog.[1,2]

he purpose of the present experiments was to investigate the effect of 2-no-2-hydroxymethyl-1,3-propanediol or tris(hydroxymethyl)aminomethane IAM) on the pulmonary artery hypertension of hypercapnic acidosis. AM, when administered in proper amounts during apneic oxygenation, will arterial blood pH constant, but will not prevent the accumulation of CO_2. ler such conditions one may distinguish effects due to an excess of H^+ ions n those due to elevation of CO_2 in the form of HCO_3^-. In addition, an mpt was made to determine the mechanism of the pulmonary hypertension rved during severe hypercapnic acidosis. Indirect measurements of blood . using the direct Fick method, were made and pulmonary resistance ulated. Pressure measurements were also made in different segments of pulmonary vascular bed, in order to assess more clearly which segments e particularly affected by the pulmonary hypertension of hypercapnic losis.

METHODS

Experimental Procedure

welve healthy mongrel dogs weighing 8 to 13.5 kg. were used. Ten minutes r an intramuscular administration of 0.2 mg. of Atropine sulfate, the animal anesthetized with intravenous sodium pentothal (25 mg./kg.). The l cords were sprayed with 3 ml. of 1 per cent xylocain, the animal intubated, placed on a positive pressure ventilator driven mechanically at a rate of o 18 per min. with a ratio of inspiration to expiration of 1 to 2. The ven- or was supplied with 100 per cent oxygen. Following this maneuver, 0.5 per kg. of succinylcholine was administered intravenously. Muscular xation was maintained throughout the experiment with fractionated s of succinylcholine, an average total dose of 2 mg./kg. being adminis- d. Polyethylene catheters were introduced into the femoral artery and to measure blood pressure and administer fluid. A cardiac Cournand eter was introduced through the jugular vein into the pulmonary artery. ome experiments, a second Cournand catheter was placed into a "wedge tion" to measure the pulmonary "capillary" pressure. In some experi- ts a third catheter was also introduced by way of the femoral artery into left ventricle and, when possible, into the left auricle and the pulmonary vein. These catheters were connected to strain gauges and to a multicha photographic (Telmco) recorder. Electrocardiogram in the standard was also recorded. After 1 hour of ventilation, the ventilator was stop and the trachea of the animal was connected through a T-tube to a Bene Roth type spirometer containing 100 per cent O_2. Valves and soda lime been removed from the spirometer, which was kept filled by an intermittent flow of O_2. O_2 uptake was continuously recorded on the spirometer trac "Apneic oxygenation" was maintained for 30 min. The dogs seldom quired any further administration of succinylcholine to maintain respira paralysis during apnea. Mechanical ventilation was resumed after ap until the animal started breathing spontaneously, usually within 20 Blood samples and records of systemic, arterial, pulmonary, and "capill pulmonary pressures were taken: immediately before the onset of ap after 15 min. of apnea, and after 30 min. of apnea.

In 6 experiments at the onset of apnea a 0.33 *M* intravenous infusio THAM was administered at the rate of 1 ml./kg./min., a quantity dee sufficient to buffer the estimated amount of CO_2 produced by the ani Thirty mEq of NaCl were added to the solution to balance the Na^+ and ions lost in the urine, making the solution administered slightly hypert (330 mOsm.).

In a third series of experiments, 6 dogs were maintained in the same ex mental conditions of apneic oxygenation during 45 min. After 30 min apnea, they were given a 0.66 *M* infusion of THAM at the rate of 0.66 mm kg./min.

Analytical Procedure

Arterial blood *p*H was measured anaerobically with a glass capillary elect thermostated at 38° C. (Radiometer). The maximum variation for the va obtained in duplicate determinations was ±0.01 *p*H unit. Blood O_2 and b CO_2 content were measured by the Van Slyke manometric method; pla CO_2 content was calculated from the nomogram of Van Slyke and Send $PaCo_2$ and HCO_3^- were calculated by the Henderson-Hasselbalch equat Oxygen uptake was measured directly over periods of 10 min. from the s of spirometer tracing, corrected for CO_2 accumulation in the lung, and pressed in ml./kg./min., S.T.P.D. Cardiac output was measured accor to the direct Fick method. Pulmonary resistance was calculated accordin the formula:

$R = P \times 1332/F$ where:
R = resistances (dynes/sec./cm.$^{-5}$)
P = mean pulmonary arterial pressure (mm. Hg), when total pulmo resistance was calculated
= difference in mm. Hg between mean pulmonary arterial and "capill pressures, when "arteriolar pulmonary" resistance was calculated
F = pulmonary blood flow (ml./sec.)
1332 = factor correcting millimeters of Hg to dynes/cm.$^{-5}$.

Results

*Control Animals**

cid base balance, oxygen uptake and arterial oxygen saturation. After 30 . of apnea, PaCO_2 rose from 30 (range 26 to 32) to 152 mm. Hg (range 141 55), total plasma CO_2 from 16.7 (range 15.1 to 19.3) to 30.9 mmole/l. ge 27.7 to 35.3) and plasma HCO_3^- from 15.8 (range 14.2 to 19.1) to 24.9 ole/l. (range 22.8 to 27). There was a fall in arterial *p*H from 7.38 (range to 7.45) to 6.85 (6.80 to 6.99). Plasma HCO_3^-/H_2CO_3 ratio fell to 4. Ar- l oxygen saturation varied between 98 and 91 per cent. Oxygen uptake ys decreased: from 9.57 ml./kg./min. (range 8.82 to 9.87) between 0 and min., to 8.79 ml./kg./min. (range 8.11 to 9.01) between 20 and 30 min., this fall was not significant.

emodynamic changes. After 30 min. of apnea, heart rate fell from 202 ge 191 to 229) to 105/min. (range 99 to 131), and cardiac output decreased n 2.03 (range 1.86 to 2.11) to 1.34 l./min. (range 1.21 to 1.59). Mean rial systemic pressure rose from 98 (range 82 to 121) to 129 mm. Hg (range to 153). Mean pulmonary artery pressure increased from 19 (range 12 to to 26 mm. Hg (range 19 to 29) after 15 min. and to 29 mm. Hg (range 22 5) after 30 min., systolic pressure rising from 28 (range 18 to 30) to 40 . Hg (range 27 to 50), diastolic pressure rising from 14 (range 7 to 19) to nm. Hg (range 13 to 31). Pulmonary "capillary" pressure increased from ange 4 to 9) to 16 (range 12 to 18 mm. Hg. Left ventricle telediastolic ssure increased from 7 (range 5 to 9) to 10 mm. Hg (range 7 to 13). Left icular pressure increased from 5 (range 2 to 6) to 7 mm. Hg (range 5 to 9). r 30 min. of apnea, pulmonary venous pressure was 1 mm. Hg (range 0).

alculated systemic resistance increased from 3858 (range 3021 to 4243) to 3 dynes/sec./cm.$^{-5}$ (range 6924 to 8531) after 30 min. of apnea. Calculated l pulmonary resistance increased from 551 (range 482 to 603) to 1313 es/sec./cm.$^{-5}$ (range 1283 to 1524). Calculated arteriolar pulmonary re- nce increased from 443 (range 392 to 483) to 775 dynes/sec./cm.$^{-5}$ (range to 831).

Animals Treated with THAM during 30 Minutes of A.O.†

cid base balance, O_2 uptake and arterial oxygen saturation. After 30 min. of ea, PaCO_2 rose from 29 (range 24 to 32) to 58 mm. Hg (range 49 to 69), l plasma CO_2 from 14.4 (range 13.1 to 18.2) to 32 mmole/l. (range 27 to) and plasma HCO_3^- from 13.5 (range 12.1 to 17.3) to 30.3 mmole/l. (range to 38.2). Arterial *p*H was maintained between 7.32 (range 7.29 to 7.38) 7.35 (range 7.30 to 7.41). The plasma HCO_3^-/H_2CO_3 ratio remained in physiological limits of 18 to 1. Arterial oxygen saturation was 98 per cent r 30 min. of apnea. Oxygen uptake was maintained constant: 8.53 ml./ min. between 0 and 10 min. (range 8.01 to 9.03), 8.55 ml./kg./min. be-

FIGURES 1 and 2.
FIGURES 2 and 3.

tween 10 and 20 min. (range 8 to 9.12); 8.50 ml./kg./min. between 20 an min. (range 7.99 to 8.91).

Hemodynamic changes. Heart rate decreased from 196 (range 168 to to 142/min. (range 129 to 163) after 30 min. of apnea. Cardiac output maintained constant: 1.95 l./min. (range 1.72 to 2.15) at the start of the periment, 2.01 l./min. (range 1.81 to 2.31) at the end of the apnea.

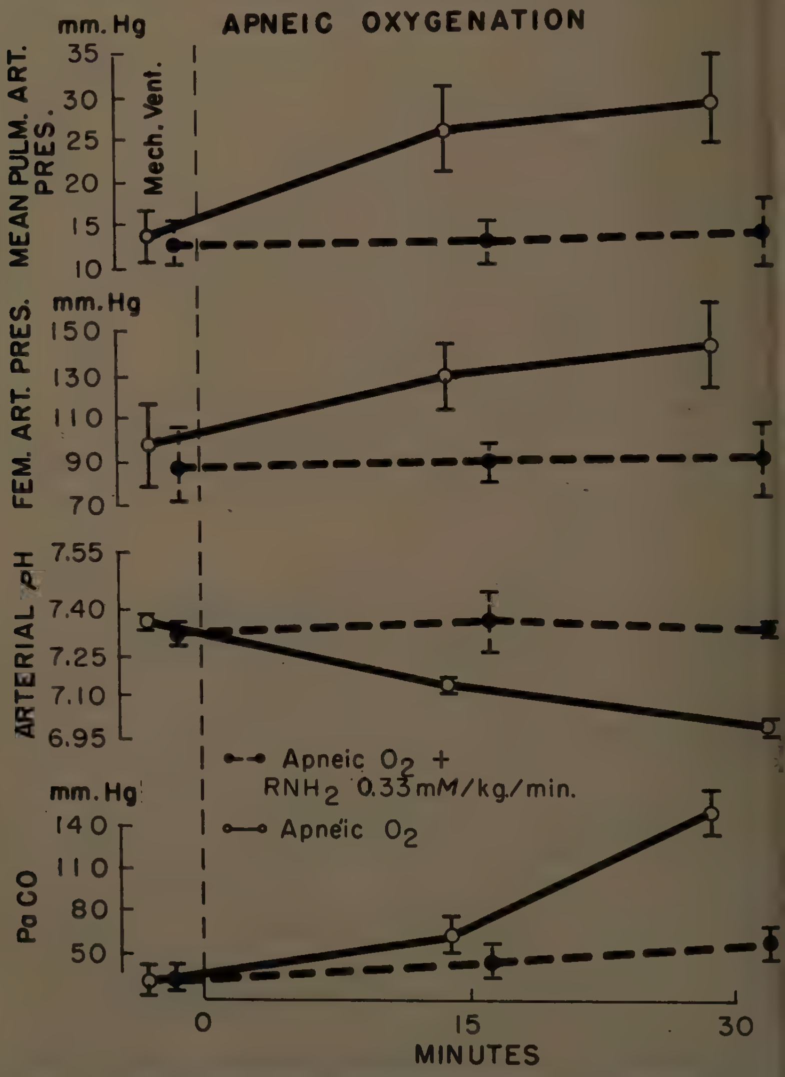
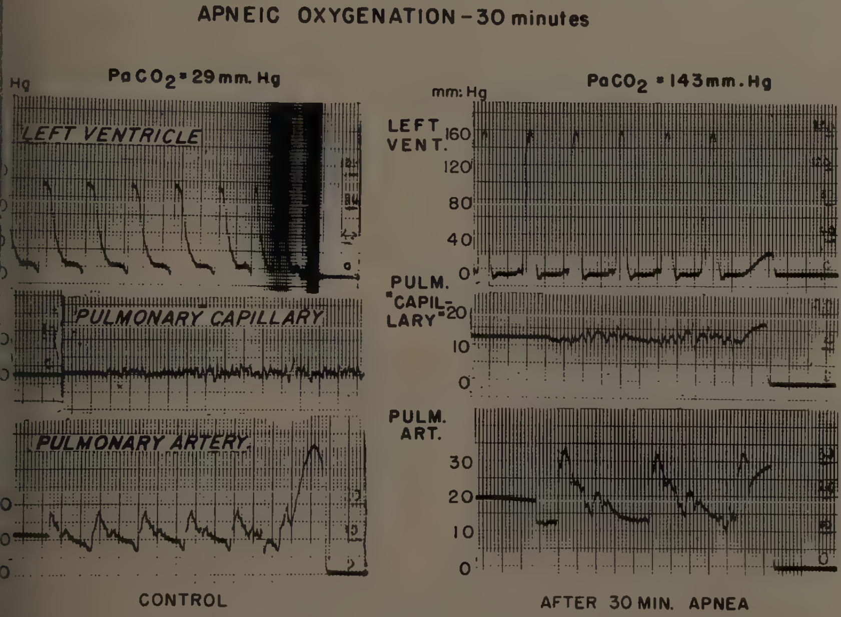
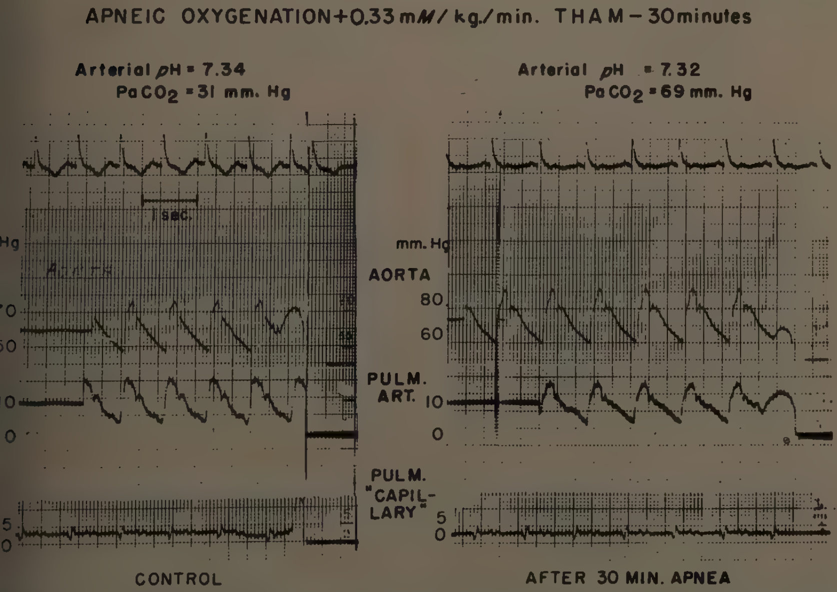


FIGURE 1. Effects of intravenous administration of THAM (0.33 *M* solution, 1 ml min.) in 6 dogs during 30 min. of apneic oxygenation. While marked increases in arterial and pulmonary artery pressures are observed during a similar period of apne control animals (solid line), in the treated animals there is no significant change in pre or *p*H, and average $PaCO_2$ does not rise above 58 mm. Hg.



IGURE 2. Records of left ventricle, pulmonary “capillary” and pulmonary artery pres-
s in a dog, before and after 30 min. of apneic oxygenation.



IGURE 3. Records of electrocardiogram, aortic, pulmonary artery, and pulmonary
illary” pressures, before and after 30 min. of apenic oxygenation in a dog treated with
AM.

systemic pressure was 92 mm. Hg (range 82 to 133), not significantly differe
from the control level: 88 mm. Hg (range 71 to 121). Mean arterial puln
nary pressure was maintained constant: 18.5 mm. Hg at the onset of apn
(range 12 to 21.5), 20.5 mm. Hg at the end of the experiment (range 12 to 2
systolic pressure varying between 30 (range 19 to 33) and 35 mm. Hg (ran
20 to 40), and diastolic pressure between 14 (range 8 to 17) and 16 mm.
(range 9 to 19.5). Mean "capillary" pulmonary pressure, after 30 min.
apnea, was 8 mm. Hg (range 3 to 12), similar to the control, 7 mm. Hg (ran
2 to 11). Left auricle, telediastolic left ventricle and venous pulmonary pr
sures did not change and were always below 7 mm. Hg throughout the expe
ment (range 2 to 7).

Calculated systemic resistance was 3271 dynes/sec./cm.$^{-5}$ at the onset
apnea (range 2671 to 3821) and at 3296 dynes/sec./cm.$^{-5}$ at the end of
experiment (range 2929 to 3741). Calculated total pulmonary resista
varied between 614 (range 531 to 703) and 636 dynes/sec./cm.$^{-5}$ (range 604
713). Calculated arteriolar pulmonary resistance varied between 471 (ran
399 to 533) and 497 dynes/sec./cm.$^{-5}$ (range 424 to 553).

Animals Treated with THAM for 15 Minutes Following 30 Minutes of A.

After 30 min. of apnea, the arterial *p*H had fallen from 7.32 (range 7.28
7.39) to 6.95 (range 6.80 to 7.11); the PaCO$_2$ increased from 31 (range 27
39) to 154 mm. Hg (range 139 to 183); and the HCO_3^-/H_2CO_3 ratio droppec
4.3. In the same period, the mean systemic arterial pressure rose from
(range 78 to 129) to 140 mm. Hg (range 121 to 163), the mean pulmona
arterial pressure rose from 17 (range 14 to 19.5) to 24 mm. Hg (range 20 to
and the mean "capillary" pulmonary pressure from 6 (range 2 to 8) to 16 n
Hg (range 12.5 to 19).

At the end of 45 min. of apnea, after the animals had received 0.66 mmo
kg./min. of 0.6 *M* THAM during the last 15 min., the arterial *p*H was 7
(range 7.27 to 7.40), the PaCO$_2$ was 63 mm. Hg (range 53 to 82), the me
systemic arterial pressure decreased to 80 mm. Hg (range 69 to 103), the me
pulmonary arterial pressure decreased to 14 mm. Hg (range 8 to 17) and "c
illary" pulmonary pressure to 4 mm. Hg (range 0 to 8).

DISCUSSION

Pulmonary hypertension during hypercapnic oxygenation of A.O. affe
both the pulmonary artery and the pulmonary capillary vessels, and is of c
siderable magnitude. After 30 min. of "apneic oxygenation" a 52 per c
rise in mean pulmonary arterial pressure is observed, whereas the mean "c
illary" pulmonary pressure doubles. This hypertension appears early and
systemic hypertension is already significant after 5 min. of apnea. It is
pecially marked in the pulmonary capillary. At the end of apnea, the press
gradient between pulmonary artery and "pulmonary capillary" increases fr
11 to 13 mm. Hg and the

$$\frac{\text{pulmonary artery pressure}}{\text{pulmonary ``capillary'' pressure}}$$

---

* FIGURE 4.

io decreases from 2.37 to 1.82. This hypertension in the capillary circula- n does not result from any hemodynamic changes occurring in the systemic culation. Left ventricle telediastolic, left auricle, and pulmonary venous essures do not change during the 30 min. of apnea.

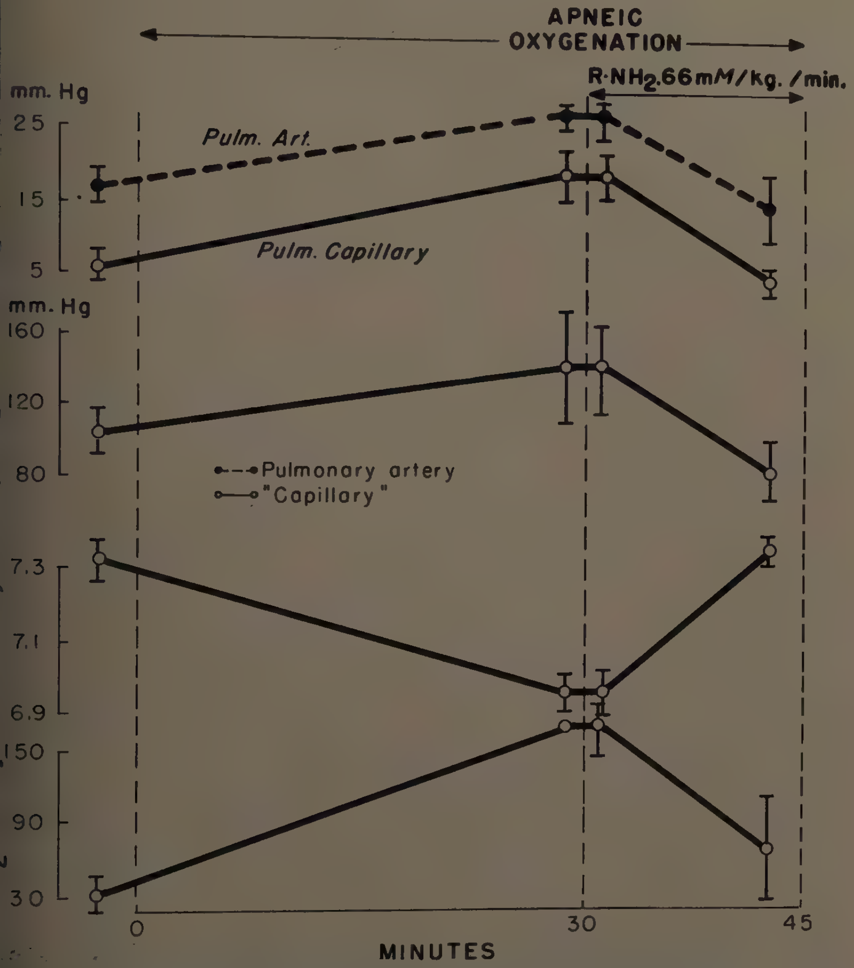


Figure 4. Effects of intravenous administration of THAM (0.66 M solution, 1 ml./kg./ .) in 6 dogs during the last 15 min. of a 45-min. period of apneic oxygenation. All varia- s are restored to preapneic control levels, except $PaCO_2$, which decreases from 154 to 63 . Hg.

What is the mechanism of this hypertension increase in pulmonary vascular istance or increase in flow? Cardiac output, measured by the direct Fick thod, is decreased after 30 min. of apnea. A similar finding was reported Holmdahl.[1] However, Nahas and L'Allemand,[2] using the dye dilution thod for measuring cardiac output, reported a rise in cardiac output and ncluded that the increase in pulmonary artery pressure could be accounted for by the increase in blood flow. In the present series of experiments, t increase of "total" calculated pulmonary resistance as well as of calculat arteriolar pulmonary pressure which were observed, had doubled at the end apnea. However, when THAM was administered, no such changes were o served, since neither cardiac output nor pulmonary artery pressure did va from the control. Furthermore, an infusion of 0.66 *M* THAM, after 30 mi of apneic oxygenation, which restored arterial blood *p*H to normal, in the pre ence of a continued CO_2 accumulation, was accompanied by a fall of pulm nary artery and "capillary" pressures.

We interpret these observations as an indication that severe hypercap produces an increase in pulmonary vascular resistance. The mechanism this increase appears to be related to the increase in free CO_2 in the blood, a not to CO_2 accumulation. This pressure increase is not observed when *p*H maintained constant, or when *p*H is restored to normal. Whether the rise H^+ ion is the direct cause of the increase in pulmonary vascular resistance, simply triggers a nervous or hormonal stimulus, is still to be determined. T great increase of catecholamine plasma levels during hypercapnia is well know After 30 min. of apneic oxygenation, plasma epinephrine rises as high as μg/l. and plasma norepinephrine as high as 28 μg/l.[4] However, the pulmona vessels are known to be little influenced by catecholamine injection.[5] Ho ever an increase in H^+ ion could still have a direct effect on the sympathe nerves of the vessels.

The effects of inhaling carbon dioxide mixtures on the pulmonary circulati have been investigated by some authors with inconsistent results.[6,7] Be *et al.*,[8] on the basis of observations on isolated vascular segments, Binet a Bourliere,[9] and Duke,[10] on special animal preparation, have suggested that t breathing of air enriched with CO_2 may elicit vasoconstriction. The effe of inhaling 5 per cent carbon dioxide in air on the pulmonary circulation we studied in humans by Fishman *et al.*[11] In the 5 control subjects with an av age increase in arterial $PaCO_2$ of 6 mm. Hg, both pulmonary arterial flow a blood pressure remained unchanged. In 10 patients with chronic pulmona emphysema with a similar increase in arterial $PaCO_2$, there was a 14 per ce increase in cardiac output and a rise in pulmonary arterial mean pressure 4 mm. Hg. However the authors did not measure pulmonary vein pressu in their experiments, nor did they account for changes in intrapleural pressur Also, the increase in $PaCO_2$ of 6 mm. Hg was small when compared to th observed in the present experiments. The limited rise in $PaCO_2$ in the expe ments of Fishman *et al.* could be explained by the compensatory hyperventi tion that the subjects presented when breathing elevated CO_2 gas mixtures.

Summary and Conclusions

The hypercapnic acidosis of apneic oxygenation (A.O.) causes a pulmona hypertension that appears early and is particularly important in the "ca lary" pulmonary bed. It seems that this hypertension is related to an increa in pulmonary resistance, which may be caused by the intensive stimulation the sympathoadrenal system which occurs during hypercapnia.

When hypercapnia is treated with THAM during A.O., there are no mea ble changes in the pulmonary circulation. Accumulation of CO_2 in the ganism to more than twice its normal value is accompanied by a normal *p*H. erefore, in hypercapnia the increase in H^+ ion concentration seems to be a jor factor in the stimulation of the sympathoadrenal system and, possibly, the resulting pulmonary hypertension.

In addition to its preventive action on the development of the pulmonary pertension of hypercapnia, THAM also corrects rapidly and completely the lmonary hypertension of acute hypercapnic acidosis, after it has developed. lministration of THAM in clinical conditions of hypercapnic acidosis may of value.

Acknowledgments

We acknowledge with thanks the suggestions of G. G. Nahas in planning e foregoing experiments and in drafting this manuscript. The help and llaboration of M. Duthil and Miss Destruel are gratefully acknowledged.

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Discussion of the Paper

G. G. Nahas: I do agree with Ligou that the pulmonary hypertension asiated with acute severe hypercapnic acidosis is due to an increase in pulnary resistance. However, I should not accept this conclusion for the same sons that he does. This increase in pulmonary resistance would be difficult prove solely on the basis of a decrease in cardiac output measured by the ect oxygen Fick method. Indeed, this method is not suitable for measurent of cardiac output under conditions of "apneic oxygenation," which is an steady state during which the respiratory exchange ratio tends towards zero. addition, cardiac output measured during apneic oxygenation by the dye ution method indicated an increase in cardiac output which actually could ount for the rise in pressures.[1] Discrepancies in cardiac output measurements by two applications of the Fick principle are not new and were empl sized and explained by Visscher at the time I was working in his department

Furthermore, "calculated pulmonary resistance," even on the basis of curate measurements of cardiac output, only gives a mean statistical fig for the total resistance in the vascular bed of the lung; however, one can c ceive of a situation in which certain areas in the lung would present an incre in resistance, while others would present a decrease in resistance, althou total blood flow through the lung would remain the same, being merely red tributed from an area of greater to one of lower resistance. Such conditi may prevail in apneic oxygenation. The significant fall in arterial O_2 satu tion, which is present after 30 min. of apnea, can only be explained by shunt of large amounts of venous blood.

The fact that during THAM administration no change in pulmonary pr sure is observed and that O_2 arterial saturation is not lowered indicates, Ligou points out, that THAM prevents both the stimulation of the sympat adrenal system and the rise in H^+ ion concentration in plasma and body flu which accompanies the pulmonary hypertension of hypercapnic acido However, one should, in addition, consider the possibility that THAM p duces a vasodilatation of the segments of the pulmonary vascular bed, wh are constricted when hypercapnic acidosis is present. The constancy of p monary artery and pulmonary capillary pressures during THAM infusio particularly remarkable, when one considers the large hypertonic fluid l (1 ml./kg./min.) that is administered to the animal. Constancy of cardiac o put measured by the direct oxygen Fick method is more difficult to interp under such conditions of fluid load which usually produce an increase in card output.

It should also be emphasized that the increase in $PaCO_2$ occurring afte min. of apneic oxygenation is on the average 30 mm. Hg, which is similar the increase in $PaCO_2$ observed after 30 min. of A.O. and simultaneous TH administration. In the first instance, after 5 min. of A.O., pulmonary art pressure is markedly increased, while it is not changed after 30 min. of A associated with THAM infusion.

These observations would also indicate that in severe decompensated e physema, where $PaCO_2$ may be higher than 80 mm. Hg, a marked increase pulmonary resistance may be present and may contribute to the severity this clinical condition.

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THE EFFECTS OF ACUTE ALKALOSIS AND ACIDOSIS ON THE PULMONARY CIRCULATION*

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t is generally agreed that the rise in pulmonary arterial pressure during ite hypoxia is a consequence of pulmonary vasoconstriction. The mecha-m by which this effect is produced is still poorly understood. One potential tor involves the effects of the by-products of glycolytic metabolism during iods of O_2 lack.[1,2] Recently, Liljestrand called attention to the production inordinate quantities of lactic acid by the isolated lung preparation during ite, severe hypoxia; he attributed the pulmonary arterial pressor response to oxia to this acid.[3] Unfortunately, several considerations limit the transfer this hypothesis from the isolated lung to the intact animal: (1) lactic acid els do not increase in the blood during moderate degrees of hypoxia suffi-nt to increase pulmonary arterial pressure in the intact animal,[4,5] and (2) pulmonary arterial pressor response to hypoxia is generally attended by temic alkalosis, rather than acidosis. Furthermore, the few studies that re attempted to establish the role of the hydrogen ion concentration in the ulation of the pulmonary circulation have been inconclusive.[6,7]

he present study undertook: (1) to examine the role of lactic acid in the monary arterial pressor response to acute hypoxia in man; (2) to investigate effects of induced acidosis on the pulmonary circulation of the intact dog; l (3) to distinguish the pulmonary vascular effects of the hydrogen ion from se of its associated anions.

Methods and Results

he possibility that either intracellular or extracellular acidosis is involved the pulmonary arterial pressor response to hypoxia was tested by the in-ion of alkalis during acute hypoxia in man. Acute hypoxia was induced in nanesthetized human subjects by exposure to 12 per cent O_2 in N_2, before l after the intravenous infusion, during a period of 25 min., of either 300 ml. .3 *M* tris-hydroxymethyl-aminomethane (THAM) or of 0.4 *M* sodium bi-bonate. Pulmonary arterial blood pressures were measured by means of a diac catheter, and systemic blood pressures by an indwelling brachial arterial dle; pulmonary blood flow was measured by the direct Fick principle. The ılts obtained in the 5 subjects infused with THAM are summarized in URE 1. It may be seen that the pulmonary arterial pressor response to te hypoxia was not modified by the THAM, despite marked increases in the erial blood *p*H. Virtually identical results were obtained in the 4 subjects received sodium bicarbonate.

This investigation was supported in part by Research Grant H-2299, C3 from the Na-al Institutes of Health, Public Health Service, Bethesda, Md., with additional support the New York Heart Association, New York, N.Y., and the American Heart Associa-, New York, N.Y.

In order to induce different types of acidosis, anesthetized dogs were eit
infused with lactic or hydrochloric acid or made to breathe a 5 per cent (
mixture. The compensatory increase in ventilation that characteristic

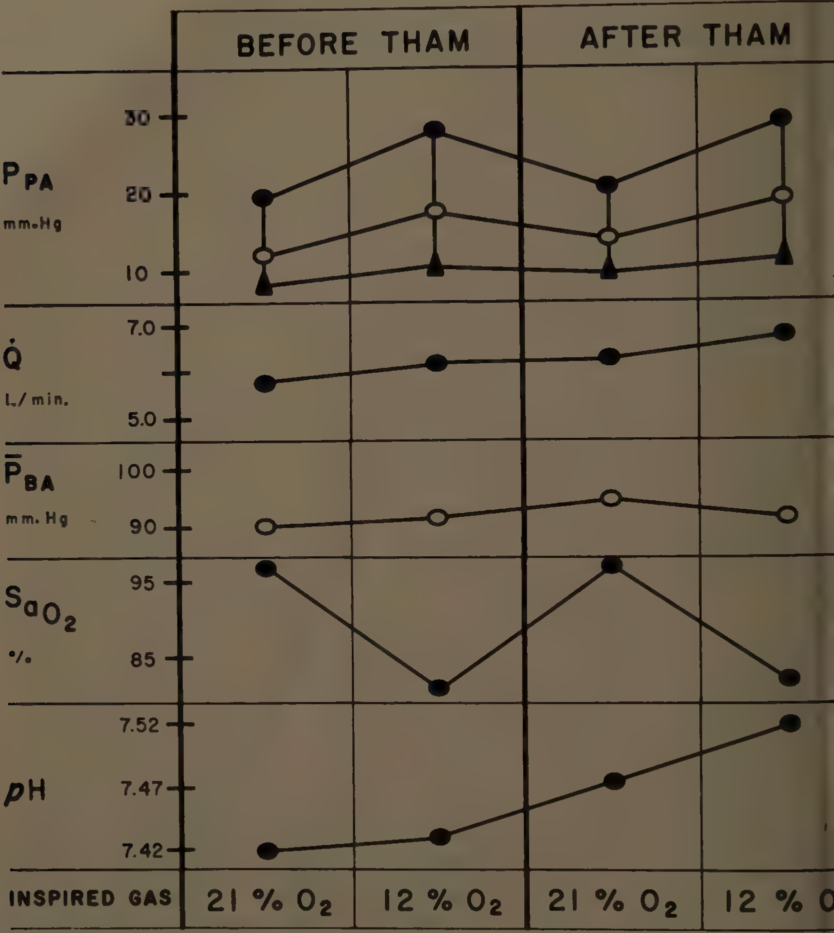
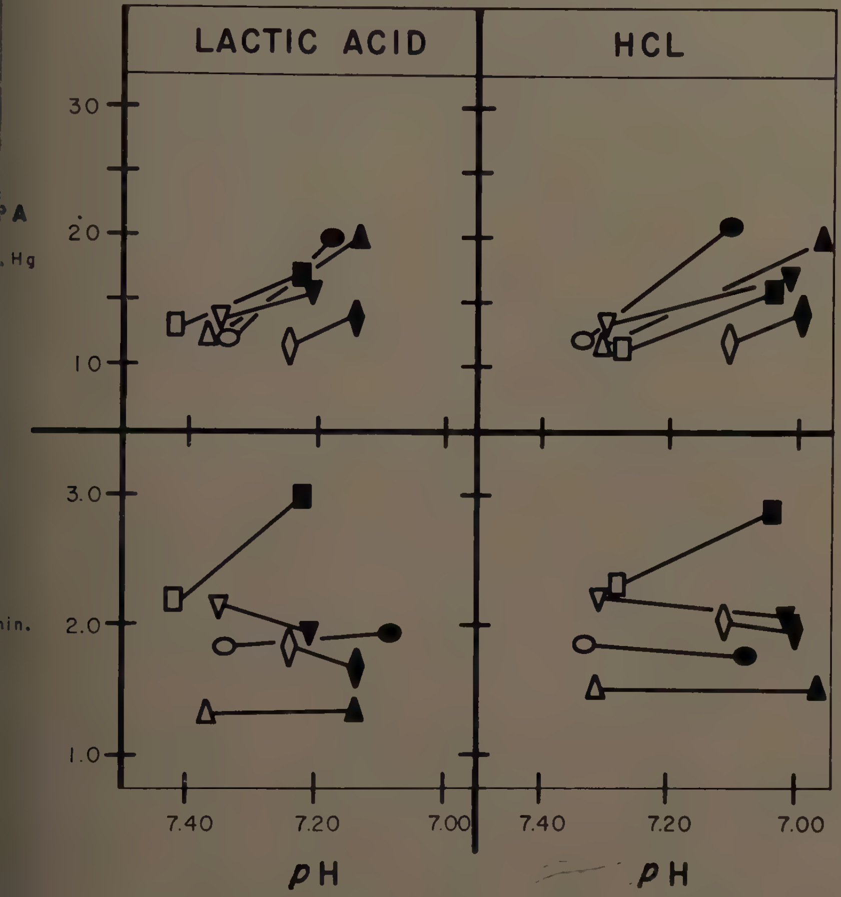


FIGURE 1. The effects of infusing 0.3 *M* THAM on the pulmonary arterial presso
sponse to hypoxia in man. Each point in this figure represents the average of the v
obtained in 5 individuals.

P_{PA} = pulmonary arterial pressure, $\dot{Q}$ = pulmonary blood flow, $\bar{P}_{BA}$ = mean bra
arterial pressure, S_{aO_2} = arterial oxyhemoglobin saturation, pH = negative log of th
drogen ion concentration of arterial blood.

occurs in spontaneously breathing subjects during acidosis was prevente
mechanically-controlled positive pressure ventilation. The results are s
marized in FIGURES 2 and 3. FIGURE 2, representing individual data on 5 c
indicates that: (1) the infusion of lactic acid (0.3 *M* at a rate of 4.0 ml./
for 15 min.) elicited an increase in pulmonary arterial pressure without

ently altering pulmonary blood flow; and (2) infusions of hydrochloric acid rate sufficient to produce the same decreases in arterial blood *p*H elicited same effects as lactic acid on the pulmonary circulation.



IGURE 2. The effects of infusions of 0.3 *M* lactic acid and of 0.3 *M* hydrochloric acid on nean pulmonary arterial pressure ($\bar{P}_{PA}$) and the pulmonary blood flow ($\dot{Q}$) of 5 anesthe- dogs. Different symbols are used to identify individual animals; in each case, the clear ols represent the values prior to the infusion; the solid symbols represent the values at f the infusion.

FIGURE 3 the effects of the metabolic acidosis produced by the infusion of c acid are compared in 3 dogs with the effects of the respiratory acidosis ced by the controlled ventilation with 5 per cent CO_2. It may be seen at comparable levels of acidosis, the pulmonary circulatory effects were ally identical. Not illustrated are the observations that acute acidosis, atter how induced, was without effect on the left ventricular systolic or tolic pressures.

Discussion

In the present study, the failure of either sodium bicarbonate or THAM modify the pulmonary arterial pressor response to hypoxia in man indica that neither extracellular nor intracellular* acidosis plays a role in this pres response. On the other hand, one consistent observation in the present stu

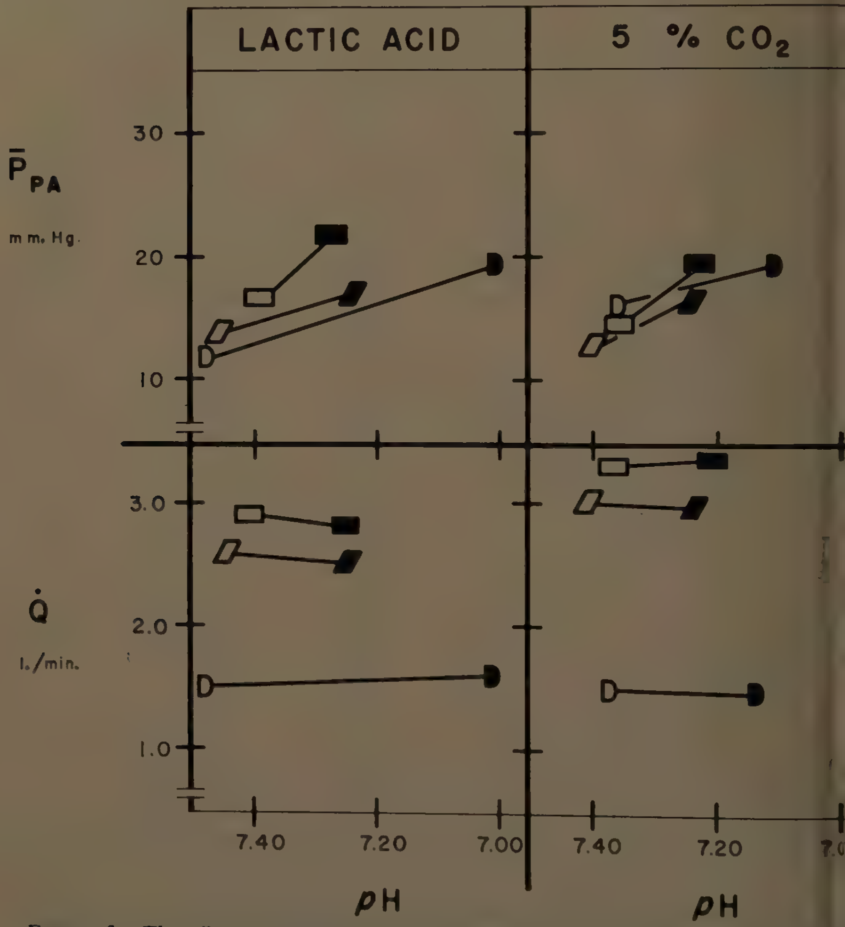


FIGURE 3. The effects of infusions of 0.3 *M* lactic acid and the breathing of 5 per CO_2 in 3 animals. The symbols and notations are the same as in FIGURE 2.

was the rise in pulmonary arterial pressure during acute acidosis. Since pulmonary blood flows and left ventricular blood pressures remained unchan the rise in pulmonary arterial pressure is attributable to pulmonary vasoc striction. Finally, the demonstration that the relationship between the crease in pulmonary vascular resistance and the decrease in arterial blood was the same, regardless of the method used to induce acidosis, suggests t

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* Nahas, G. G. 1960. Personal communication.

hydrogen ion, rather than its associated anion, is responsible for the pul-
nary vasopressor response.

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EFFECTS OF 2-AMINO-2-HYDROXYMETHYL-1,3-PROPANEDIOL ON THE CENTRAL RESPIRATORY MECHANISMS IN THE CAT*

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The effect of 2-amino-2-hydroxymethyl-1,3-propanediol, an organic buff often called Tris buffer or THAM, on respiratory movements was studied decerebrate cats with brain stem transection at various levels. The respir tory responses to electrical stimulation of the pontile pneumotaxic center, t medullary inspiratory center, and to stimulation of the central cut end of t vagus nerve before and during the infusion of the amine buffer were also inves gated. These effects were correlated with changes in arterial blood *p*H, carb dioxide tension, and bicarbonate concentration, in an attempt to appraise t role of H-ions and/or carbon dioxide in the control of respiration.

Methods

Experiments were carried out in 61 cats weighing between 2.4 to 4 kg. eac Midcollicular decerebration was performed under ether anesthesia. Th animal was allowed to recover from anesthesia by breathing room air for least 1 hour, after which "denitrogenation" was accomplished by administeri oxygen through a nonreturn valve for 5 to 10 min. Respiratory movement w then recorded by connecting the tracheal cannula to a small animal spiromet writing on a kymograph or, in later experiments, to a servospirometer (Moc 350‡) on a Grass Polygraph (Model 5). Exhaled carbon dioxide was remov from the system by a soda lime absorber and oxygen was continuously added it. Arterial blood was sampled through an indwelling catheter in a femor artery. These samples were analyzed for *p*H and CO_2 content[1] and, from t resulting data, carbon dioxide tension ($PaCO_2$) and bicarbonate concentrati (HCO_3^-) were derived.[2] With a Harvard infusion pump, 2-amino-2-hydrox methyl-1,3-propanediol in 0.8 *M* solution was infused into a femoral vein at rate of 0.3 to 0.5 mmole/kg./min. and occasionally up to 1 mmole/kg./mi To produce rapid changes, 5 mmole/kg. of the buffer was sometimes admin tered as a single injection.

To study the effect of the amine buffer on pontile and medullary anima the brain stem was transected at the appropriate levels. With rostral pont transection and bilateral vagotomy, the respiratory pattern was apneust In medullary animals, respiration was generally periodic and gasping in natur Amine buffer was usually infused when the new pattern of respiratory mo ment was well established.

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† Postdoctoral trainee, Department of Anesthesiology, Division of General Medi Sciences, Public Health Service, Bethesda, Md., under Grant No. 2G-56.

‡ Custom Engineering and Development Co., St. Louis, Mo.

Midcollicular decerebrate, vagotomized preparations were used to study spiratory responses to electrical stimulation of the pontile pneumotaxic nter, the medullary inspiratory center, and the central cut end of the vagus rve both before and during infusion of the amine buffer. The stimulating ectrode (concentric) was placed in the respective areas in the brain stem with e aid of a stereotaxic instrument. Stimulus currents were rectangular pulses 50 cps frequency, 2 msec. duration, and varied intensity, derived from a rass Stimulator (Model S4C) through a stimulus isolation unit. For central gal stimulation, a Palmer bipolar electrode was used, and both pulse fre- iency and intensity were varied.

Results

Effect on respiratory movements of animals with brain stem transections. In idcollicular decerebrate preparations with eupnea, infusion of the amine iffer at the rate of 0.15 to 0.5 mmole/kg./min. decreased respiratory ampli- de within 2 to 3 min. The respiratory rate was usually but not consistently duced (FIGURE 1). Infusion at a higher rate (0.6 to 1 mmole/kg./min.) sulted in progressive diminution of both amplitude and rate of respiration d, finally, in apnea. Arterial pH, CO_2 content, and HCO_3^- concentration increased. The $PaCO_2$ decreased initially but, as the hypoventilation or nea persisted, it gradually increased, frequently to levels higher than that the control. Ventilation recovered within minutes after the infusion was scontinued. Arterial pH usually returned toward control, although the CO_3^- concentration and $PaCO_2$ remained elevated.

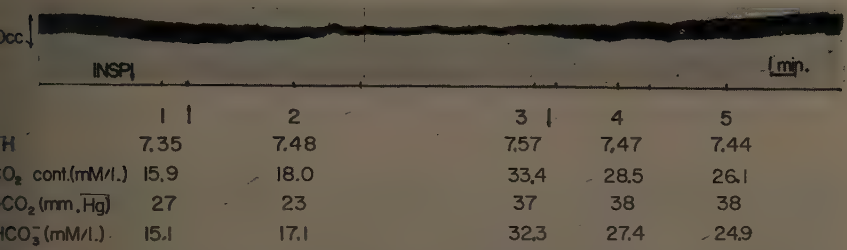
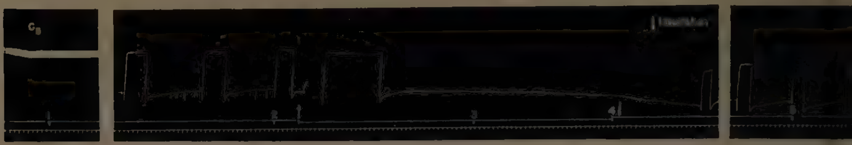


FIGURE 1. Cat T36, 3.3 kg. Midcollicular decerebrate, vagotomized. Servospirometer cing; inspiration downward. Between arrows, 0.8 *M* THAM solution was infused I.V. a rate of 0.3 mmole/kg./min. Respiratory amplitude was reduced, rate changed from 21/ n. to 19/min. Changes in arterial blood pH, CO_2 content, PCO_2, and HCO_3^- concentration re as indicated.

Ablation of the rostral pons and vagotomy usually resulted in apneustic spiration. After a short period of expiratory apnea, there was a prolonged spiratory spasm (apneusis) followed by alternating expiratory and inspira- ry spasms (apneustic breathing). The onset of apneusis and apneustic eathing was accompanied by a steady decrease in arterial pH and increase $PaCO_2$.[4] Infusion of the amine buffer at a rate of 0.3 to 0.5 mmole/kg./min. ring apneustic breathing greatly prolonged the duration of apneusis and celerated the apneustic cycling. Arterial pH and HCO_3^- concentration se, and $PaCO_2$ fell (FIGURE 2).

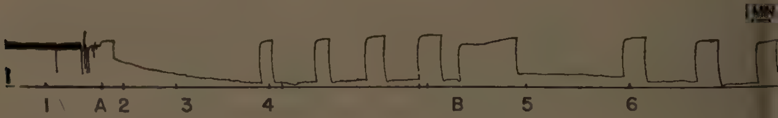
When 5 mmole/kg. of the amine buffer was given as a single injection durir the inspiratory spasm, apneusis was promptly abolished, returning after a fe minutes, at which time the $PaCO_2$ was usually in the range of 45 mm. H (FIGURE 3).

Upon ablation of the pons, there was usually a transient tachypnea, aft which the respiration became gasping in nature and regular in respect to ra



	1	2 ↑	3	4↓	5
pH	7.345	6.887	7.291	7.291	7.125
CO_2 cont (mM/l)	19.06	23.26	36.57	42.52	39.92
pCO_2 (mm. Hg)	34.1	108.5	73.6	85.5	114.4
HCO_3^- (mM/l)	18.03	20.00	34.35	39.95	36.48

FIGURE 2. Cat T5, 3.7 kg. Spirometer tracings; inspiration downward. Time mark, sec. Left panel, midcollicular decerebrate, eupnea. Between left and middle panels, rost pons ablated, vagotomized; note apneustic breathing with respiratory acidosis. Betwe arrows 0.8 *M* THAM solution was infused I.V. at a rate of 1 mmole/kg./min. for the fi minute, and then 0.3 mmole/kg./min. thereafter. Note immediate, transient reduction amplitude of apneusis and later prolongation of apneusis. Respiratory acidosis was partia reversed. Right panel, 15 min. after THAM infusion. Preinfusion apneustic cycling sumed. $PaCO_2$ returned to the previous high value.

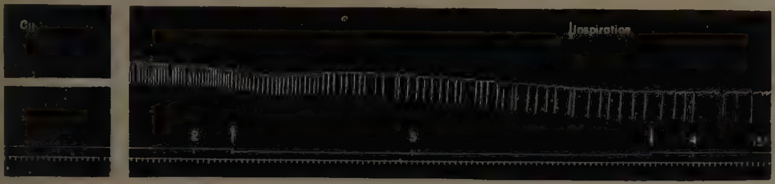


	1	2	3	4	5	6
pH	7.30	7.13	7.06	6.99	7.52	7.25
CO_2 cont. (mM/l.)	15.2	14.8	17.3	20.9	37.1	37.2
pCO_2 (mm. Hg)	30	42	58	80	42	76
HCO_3^- (mM/l.)	14.4	13.7	16.1	19.1	33.5	33.2

FIGURE 3. Cat A15, 3.6 kg. Servospirometer tracing; inspiration downward. At po *1*, eupnea after rostral pontile transection. At point *A*, bilateral vagotomy. Initial a neusis occurred at point *2* with a $PaCO_2$ of 42 mm. Hg. Apneustic cycling was initiated point *4* with a $PaCO_2$ of 80 mm. Hg. At point *B*, 5 mmole/kg. of THAM (1.6 *M* solution) v given I.V. Note immediate abolition of apneusis, which reappeared at point *5*, when Pa(was again 42 mm. Hg. Apneustic cycling resumed at point *6*, $PaCO_2$, 76 mm. Hg.

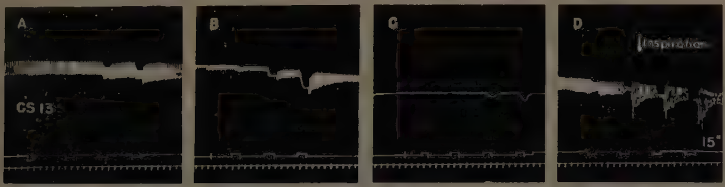
and amplitude. The predominant effect of infusing the amine buffer at a ra of 0.3 to 0.5 mmole/kg./min. was to decrease the rate. Initially the amplitu decreased minimally, but then gradually increased, frequently to a magnitu slightly greater than that of the control. Changes in arterial *p*H, HC(concentration, and PCO_2 paralleled those in midcollicular preparations (FIGU 4).

Effects on respiratory responses to electrical stimulation of the pontile pn motaxic center, the medullary inspiratory center, and the central cut end of vagus nerve. Electrical stimulation of a circumscribed area in the dorsolate icular formation at the level of the isthmus of the pons usually elicited piratory acceleration and inspiratory shift (FIGURE 5*A*).[5] During infusion the amine buffer, the acceleratory response was reduced or abolished, de-



	1	2	3	4
	7.345	7.095	7.315	7.260
cont.(mM/l.)	13.95	9.06	20.78	26.99
O_2 (mm.Hg)	24.9	27.7	40.0	58.0
O_3^- (mM/l.)	13.20	8.23	19.59	25.25

FIGURE 4. Cat T11, 3.5 kg. Spirometer tracings; inspiration downward. Time mark, sec. Left panel, midcollicular decerebrate, eupnea. Right panel, after removal of pons, iodic gasping. Between arrows, 0.8 *M* solution of THAM was infused I.V. at a rate of mmole/kg./min. Note change in rate of gasping. Amplitude was reduced slightly at first gradually increased in magnitude. Blood *p*H and HCO_3^- concentration was increased ing infusion but $PaCO_2$ was elevated.



	CONTROL	THAM 0,41 mM./kg./min.	THAM 0.82 mM./kg./min.	RECOVERY
*p*H	7.273	7,344	7.564	7,437
CONTENT (mM/l.)	11.46	14,16	23,86	20,37
CO_2 (mm.Hg)	24,4	25,4	27,4	29,8
CO_3^- (mM/l.)	10,93	13,40	23,04	19.47

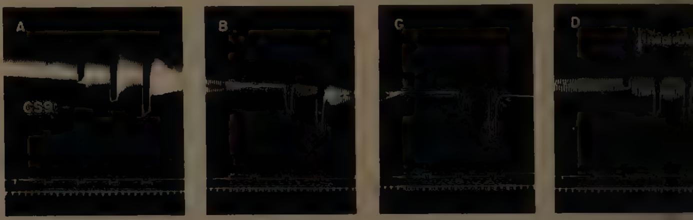
FIGURE 5. Cat S13, 2.4 kg. Spirometer tracings; inspiration downward. Midcollicular erebrate, vagotomized. Pneumotaxic center stimulation. Panel *A*, control. Parame- of stimulus current: pulse frequency, 50 cps; pulse duration, 2 msec.; voltages, 5, 6 and . Panel *B*, during infusion of THAM, 0.41 mmole/kg./min. Same parameters. Ac- ratory response abolished; inspiratory shift persisted. Panel *C*, during infusion of THAM, mmole/kg./min. Parameters: pulse frequency and duration, same as in panel *A*; volt- , 5, 6, 7 and 8 v. Threshold for inspiratory shift elevated from 6 to 7 v. No acceleratory onse. Panel *D*, recovery, 30 min. after infusion. Parameter same as in panel *C*. Ac- ratory response partially recovered.

ding on the rate of infusion and the level of ventilation. The inspiratory t persisted (FIGURE 5*B* and C).

he medullary inspiratory center was stimulated with currents of increasing nsity before the infusion of the amine buffer to establish the stimulus shold for inspiratory shift and the magnitude of inspiratory spasm (FIGURE

6*A*). During the infusion, the stimulus threshold for inspiratory shift w elevated but, with supraliminal stimuli, the magnitude of inspiratory spa was not significantly altered (FIGURE 6*B* and *C*).

Stimulation of the central end of cut vagus at low frequencies (10 to 25 c usually elicited respiratory acceleration and inspiratory shift similar to th observed during stimulation of the pneumotaxic center. The amine buf reduced or abolished the acceleratory response (FIGURE 7). Stimulation the central end of cut vagus at high frequencies (100 to 200 cps) caused expi tory apnea. With threshold stimulus, respiration was shallow and slc During infusion of the amine buffer, the stimulus threshold was lowered. T



	CONTROL	THAM 0.33 mM/kg/min.	THAM 0.66 mM/kg/min.	RECOVERY
pH	7.230	7.474	7.600	7.412
CO_2 CONTENT (mM/l.)	13.81	18.60	25.07	23.35
pCO_2 (mmHg)	31.7	25.1	25.5	36.1
HCO_3^- (mM/l.)	12.86	17.84	24.39	23.24

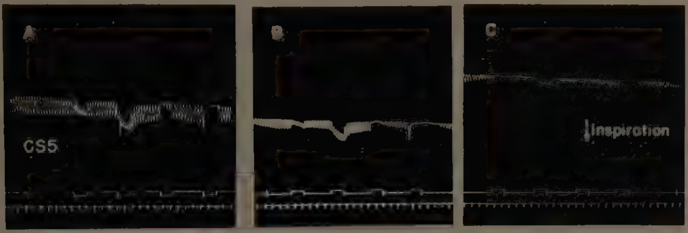
FIGURE 6. Cat S9, 3 kg. Spirometer tracings; inspiration downward. Midcollic decerebrate, vagotomized. Inspiratory center stimulation. Panel *A*, control. Parame of stimulus currents: pulse frequency, 50 cps; pulse duration, 2 msec.; voltages, 2, 3, 4 and Panel *B*, during infusion of THAM, 0.33 mmole/kg./min. Parameters: pulse frequency duration same as in panel *A*; voltages, 3, 4 and 5 v. Panel *C*, during infusion of THAM, mmole/kg./min. Parameters same as in panel *B*. Stimulus threshold for inspiratory s elevated from 3 to 4 v. Magnitude of inspiratory spasm elicited with 5-v stimulus remai unchanged. Panel *D*, recovery, 30 min. after infusion. Respiratory rate and amplitude w still less than control. Parameters: same pulse frequency and duration, voltages, 3, and 6 v. Stimulus threshold remained elevated.

inhibitory effect of the stimuli was enhanced in that the reflex apnea persis beyond the duration of stimulation (FIGURE 7).

Discussion

Administration of 2-amino-2-hydroxymethyl-1,3-propanediol induced relative alkalosis and produced hypoventilation. The respiratory effects the amine buffer are probably attributable to changes in arterial *p*H and PCO_2, which alter the activity of the central respiratory neurones. Howe it is also possible that other actions of the amine may contribute to th alterations. The amine buffer may directly depress the respiratory cen but this is unlikely, since infusion of the amine with a *p*H of 7.40 (titrated w hydrochloric acid) failed to cause significant respiratory changes in a animals. Through its hypoglycemic action (Tarail and Bennett, elsewher monograph), the amine buffer may also depress the respiratory center ectly. Studies in some of these preparations showed a marked hyper-emia (350 to 400 mg. per cent) which was not altered by the amine buffer ese dose levels.

he activity of peripheral chemoreceptors, that is, aortic and carotid bodies, doubtedly influenced by the changes in arterial pH and PCO_2. Decrease hemoreceptor activity during infusion of the amine buffer may contribute he observed respiratory changes. However, this is apparently not the ary factor, as carotid denervation and bilateral vagotomy did not influence respiratory effects of the amine buffer.



	CONTROL	THAM 0.15 mM./kg./min.	THAM 0.38 mM./kg./min.
pH	7.270	7.374	7.465
ONTENT (mM/l.)	11.98	17.13	25.39
CO_2 (mm.Hg)	25.2	28.8	34.9
ICO_3^- (mM/l.)	11.23	16.26	24.34

IGURE 7. Cat S5, 2.6 kg. Spirometer tracings, inspiration downward. Time mark, ec. Midcollicular decerebrate, vagotomized. Central vagal stimulation. Panel *A*, ol. Parameters: pulse duration, 2 msec.; pulse frequency, 10 cps; voltages, 2 and 2.5 v; pulse frequency, 100 cps; voltages, 0.4 and 1 v. Panels *B* and *C*, during infusion of M, 0.15 and 0.38 mmole/kg./min. respectively. Parameters same as in panel *A*. With requency stimuli, acceleratory responses were reduced or abolished. With high frequency uli, threshold for expiratory apnea was lowered and duration of apnea prolonged.

ypocapnia and alkalosis are known to cause cerebral vasoconstriction, decreasing cerebral blood flow.[6] Induction of relative alkalosis by the ne buffer may therefore reduce the amount of oxygen available to the n (L. C. Clark, Jr., elsewhere in these pages). However, the use of oxygen he spirometric recording after initial denitrogenation appears to rule out possibility that tissue hypoxia is a factor. In a few earlier experiments in h denitrogenation was not carried out, arterial unsaturation and gasping rred soon after the ventilation was reduced by the administration of the ne buffer. These were not observed after the procedure of denitrogenation instituted.

hus it may be concluded that the effects of the amine buffer on the central iratory mechanisms are primarily the result of changes in arterial pH and/or $)_2$. The withdrawal of the chemical drive for respiration seems to account he apparent "depression" of the respiratory center complex.

Infusion of the amine buffer appeared to decrease respiratory amplitude a
rate in all the preparations studied. In midcollicular decerebrate animals w
eupnea, the predominant effect was to decrease respiratory amplitude.
medullary animals, the major change was in respiratory rate. These findi
suggest that, in animals with an intact central respiratory mechanism,
integrating (or recruiting) mechanism is more sensitive to changes in H-
concentration and/or PCO_2 than are the rhythm regulating mechanisms. Ho
ever when the medulla is not under the influence of higher centers, the rhyt
regulating mechanisms within the substance of the medulla are still capa
of responding to changes in *p*H and/or PCO_2.

In pontile decerebrate, vagotomized animals with apneustic breathi
withdrawal of chemical drive produced a number of changes in respirat
movements. Depending upon the rate of infusion, administration of
amine buffer tended to reduce the amplitude of the inspiratory spasm. T
inspiratory spasm was always prolonged. The apneustic cycling became sl
A large dose (5 mmole/kg.) of the amine buffer abolished the inspiratory spa
which, however, reappeared when the $PaCO_2$ reached the level of appro
mately 45 mm. Hg. Furthermore, the apneustic cycling did not resume u
the $PaCO_2$ reached 76 mm. Hg (see FIGURE 3). Similar results were obtai
in other animals in which the inspiratory spasm was abolished by hypervent
tion. These observations indicate that there exist threshold values of Pa(
for the onset of apneusis and also for the initiation of apneustic cycling.
more detailed account of these phenomena will be published elsewhere.[7]
can be stated that the chemical drive is essential for the manifestation of
unmodulated, tonically active apneustic center, and that a stronger chemi
stimulus is required to activate the respiratory mechanism. These findi
also lend further support to the hypothesis that apneustic cycling is rela
to the increased $PaCO_2$.[4]

The results obtained with electrical stimulation of various components
the respiratory center may provide further information concerning the r
of carbon dioxide and/or H-ions in regulating respiration. It has been sho
previously that both the pneumotaxic center and the vagal afferents hav
facilitatory influence on the respiratory rhythm in addition to their modulat
effect on the pontile apneustic center.[3,5] During infusion of the amine buf
the facilitatory influence of these structures was reduced or abolished. On
other hand, the center appears to be more sensitive to inhibitory impulses
high-frequency central vagal stimulation). Thus the withdrawal of chemi
drive renders the center sluggish. It is also probable that the facilitat
mechanisms themselves are "depressed".

While the stimulus threshold for inspiratory shift during stimulation of
inspiratory center was elevated during the infusion of the amine buffer,
magnitude of the inspiratory spasm upon supraliminal stimulation of
inspiratory center was not changed. This may be taken to mean that wi
drawal of chemical drive requires stronger stimuli to produce a response. H
ever, the ability of the inspiratory neurones to respond to stimuli, electr
or otherwise, does not appear to be affected. This finding may be the b
for the observation that, in medullary animals, infusion of the amine bu

ot significantly alter the amplitude of gasping (see above). If gasping is ximal inspiratory effort without modulation from higher centers, changes terial pH or PCO_2, within certain limits, would not be expected to affect mplitude materially (see also S. H. Ngai, 1957[4]).

e problem of chemical control of respiration in respect to the specific n of carbon dioxide or of H-ions remains a difficult one. The results from present study are of limited value in solving this problem. Changes in ial pH and PCO_2 are only an indication of the directions of changes in the . The difference of buffering systems in various fluid compartments and diffusion of the amine buffer into the cells (Omachi *et al.*; E. D. Robin, where in this monograph) preclude any conclusion as to the relative im- ance of carbon dioxide and H-ions. Such conclusion must await further mation concerning intracellular pH and buffering system(s), specifically e of the central nervous system.

Summary

e effects of 2-amino-2-hydroxymethyl-1,3-propanediol on respiratory ements in decerebrate cats with different patterns of respiration and on ratory responses to electrical stimulation on the respiratory centers and entral vagus nerve have been investigated. It was found that the amine r produced hypoventilation in midcollicular decerebrate preparations eupnea, mainly through a decrease in respiratory amplitude. In pontile rebrate, vagotomized preparations with apneustic breathing and in medul- preparations with gasping, the predominant effect of the amine buffer is ow respiratory cycling. Electrical stimulation studies indicated that the ratory center became less responsive to facilitatory impulses and more tive to inhibitory impulses. The responsiveness of the medullary inspira- center to electrical stimuli was not depressed although its electrical stimu- hreshold was elevated. These effects of the amine buffer are probably butable to the induced alkalosis as indicated by the comcomitant changes ood pH, HCO_3^-, and PCO_2 values.

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EFFECTS OF 2-AMINO-2-HYDROXYMETHYL-1,3-PROPANEDI ON INTRACRANIAL HYPERTENSION*

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It is well established that an increase in CO_2 concentration in the blood duces a vasodilatation of the cerebral vessels[1,2] and a rise in intracranial sure.[3,4] This rise is accompanied by an increase in blood flow through brain,[5,6] but the exact mechanisms that determine these changes are still t ascertained.

The purpose of the present series of experiments was to compare the effe an organic buffer of low toxicity, 2-amino-2-hydroxymethyl-1,3-propanedi tris(hydroxymethyl)aminomethane or THAM,[7] on intracranial hypertensio hypercapnic acidosis with the effect of other agents used to relieve elev intracranial pressure. In addition, the effect of THAM on intracranial hy tension produced by intracranial compression was also studied.

MATERIAL AND METHODS

Hypercapnic Hypertension Studies

Experimental procedure. Fourteen mongrel dogs weighing 12 to 14 kg. used. The experimental procedure was the same as that described previou Pressures were measured through needles inserted into the cisterna magna into the femoral artery, and were continuously recorded during the experim A catheter was introduced into the femoral vein for administration of diffe drugs to be tested; a catheter was inserted into the bladder, and urine ou was measured during the different experimental periods. After a period hour of denitrogenation the animals underwent two 30-min. periods of ap oxygenation. Each period of apnea was followed by 30 min. of mechan ventilation, the entire experiment lasting 2 hours. During the second pe of apneic oxygenation the animals were treated with the following compou administered intravenously: 4 animals were treated with a 0.3 *M* solutio THAM at the rate of 1 ml./kg./min.; 3 were treated with a 0.3 *M* solutio THAM buffered by the addition of HCl to a *p*H of 7.40 at the rate of 1 ml./ min.; 4 dogs were treated with a 0.6 *M* solution of D-mannitol at the rat 1 ml./kg./min.; and 3 dogs were perfused at the rate of 3 ml./min. with 30 cent urea in 10 per cent invert sugar, which represents a 5.8 *M* solution.

Analytical procedure. Samples of arterial blood and cerebrospinal fluid taken simultaneously at regular intervals during the experiment. The pling was performed anaerobically. The cerebrospinal fluid sample, w ly amounted to 0.5 to 1 ml., was replaced by an equal amount of normal e. All samples were measured for pH and CO_2 content; pCO_2 and HCO_3^- calculated with the Henderson-Hasselbalch equation.

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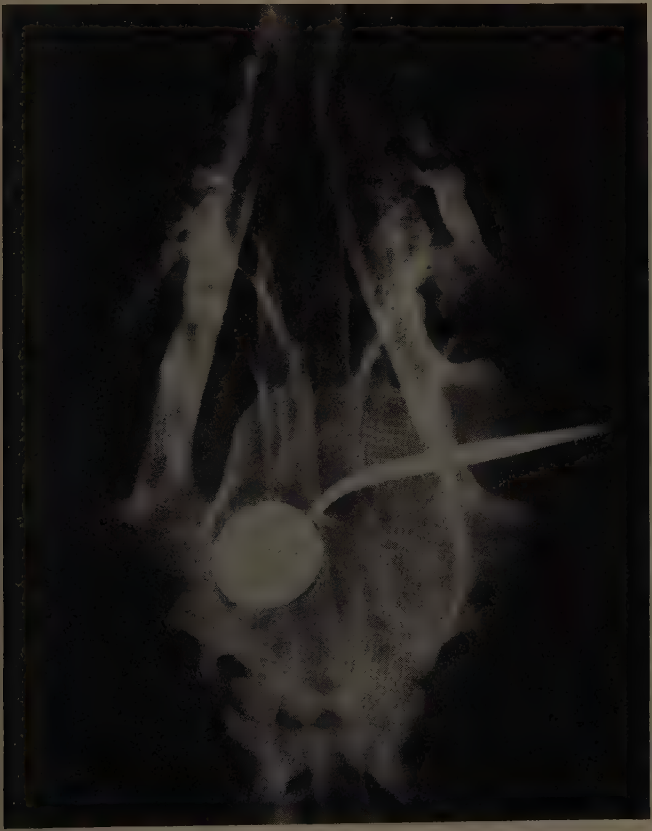
Mechanically Induced Intracranial Hypertension Studies

this series of experiments, performed on 6 dogs, increased intracranial ure was created by acute brain compression. Under general anesthesia



URE 1. Vertical submental roentgenogram of a dog skull after the intracranial balloon een inflated.

terile technique, 6 adult mongrel brachycephalic dogs were hyperventilated subjected to parieto-occipital craniotomy. A small, inflatable rubber on was inserted subdurally in contact with the posterior portion of the teral cerebral hemisphere. The free end of the catheter was buried under calp and drawn out at the time of the experiment through a skin incision. 1 to 5 weeks postoperatively, the balloon was inflated under general hesia with 4 to 5 ml. of Lipiodol, which allowed X-ray visualization RE 1). The use of an intracranial balloon, left inserted until wound healing, eliminated postoperative edema and a path for the entry of infect as experimental variables. Since the bony disk obtained by trepanation h been wired, it was impossible for the expanding balloon to bulge out of the sk The balloon was inflated in order to produce a four to sixfold increase of pr sure in the cisterna magna. This moderate pressure rise was chosen to li the untoward effects observed when greater pressure increases are acutely plied.[9] At this point the dogs were divided into 2 groups: 3 animals were p alyzed with succinylcholine chloride and mechanically hypoventilated, whil others were adequately ventilated.

RESULTS

Intracranial Hypertension of Hypercapnic Acidosis

During the first period of apneic oxygenation, in all animals, average cereb spinal fluid pressure rose from 48 mm. H_2O (range 16 to 100) to 203 mm. H (range 86 to 350) after 15 min., and to 206 mm. H_2O (range 70 to 320) af 30 min. Mean arterial blood pressure rose from 129 mm. Hg (range 90 to 1 to 149 mm. Hg (range 110 to 210) at the end of the same period. Aver arterial blood *p*H fell from 7.52 (range 7.35 to 7.68) to 6.81 (range 6.54 to 6.9 while $PaCO_2$ rose from 20 mm. Hg (range 11 to 40) to 169 mm. Hg (range to 268) and plasma HCO_3^- increased from 15.0 mEq./l. (range 10.8 to 22.1) 25.3 mEq./l. (range 18.0 to 31.5). Average cerebrospinal fluid *p*H fell fr 7.46 (range 7.21 to 7.60) to 6.79 (range 6.32 to 7.18) while cerebrospinal fl pCO_2 rose from 31 mm. Hg (range 20 to 47) to 141 mm. Hg (range 97 to 2 and cerebrospinal fluid HCO_3^- increased from 19.8 mEq./l. (range 12.8 to 2 to 20.3 mEq./l. (range 16.9 to 24.7). The urinary output was negligible.

During the second period of apneic oxygenation the results were as follo

Animals treated with THAM. Average cerebrospinal fluid pressure (TA 1) rose from 12 mm. H_2O (range 0 to 30) to 28 mm. H_2O (range 0 to 50) after min., and to 26 mm. H_2O (range 0 to 70) after 30 min. Mean arterial bl pressure rose from 123 mm. Hg (range 100 to 150) to 133 mm. Hg (range 10 156). Average arterial blood *p*H stayed within ± 0.1 U. of control value, wh $PaCO_2$ rose from 24 mm. Hg (range 15 to 34) to 58 mm. Hg (range 46 to 77) a plasma HCO_3^- increased from 15.6 mEq./l. (range 14.0 to 19.9) to 38.0 mEq (range 32.4 to 42.5). Average cerebrospinal fluid *p*H stayed within ± 0.1 of control value while cerebrospinal fluid pCO_2 rose from 33 mm. Hg (range 2 44) to 51 mm. Hg (range 43 to 76) and cerebrospinal fluid HCO_3^- decrea from 18.1 mEq./l. (range 15.5 to 20.3) to 17.3 mEq./l. (range 15.6 to 21 Urinary output increased sharply.

Animals treated with buffered THAM (pH 7.40). Average cerebrospinal fl pressure (TABLE 2) rose from 12 mm. H_2O (range 0 to 20) to 103 mm. H_2O (ra 55 to 160) after 15 min., and to 102 mm. H_2O (range 86 to 110) after 30 m Mean arterial blood pressure fell from 141 mm. Hg (range 110 to 170) to mm. Hg (range 120 to 146). Average arterial blood *p*H fell from 7.60 (ra 7.48 to 7.67) to 7.00 (range 6.95 to 7.04), while $PaCO_2$ rose from 19 mm. (range 11 to 30) to 116 mm. Hg (range 99 to 148), and plasma HCO_3^- increa from 17.1 mEq./l. (range 12.2 to 22.2) to 28.2 mEq./l. (range 26.1 to 32

TABLE 1

EFFECTS OF THAM ON HYPERCAPNIC INTRACRANIAL HYPERTENSION IN FOUR DOGS

	Arterial blood				Cerebrospinal fluid				Urine output (ml.)
	Mean pressure (mm. Hg)	pH	$PaCO_2$ (mm. Hg)	HCO_3^- (mEq./l.)	Mean pressure (mm. H_2O)	pH	pCO_2 (mm. Hg)	HCO_3^- (mEq./l.)	
Control	117* (100–138)	7.60 (7.55–7.64)	21.1 (14.1–40.4)	15.5 (13.1–22.1)	61 (40–100)	7.54 (7.48–7.59)	31.3 (25.5–47.7)	19.5 (12.9–24.1)	0
30′ A. O.†	122 (110–138)	6.87 (6.78–6.92)	157.0 (133.4–208.4)	26.3 (22.1–30.7)	147 (90–184)	6.93 (6.76–7.18)	117.4 (97.1–169.5)	19.9 (17.0–24.8)	4 (2–8)
30′ M. V.‡	123 (100–150)	7.58 (7.55–7.63)	24.3 (15.7–34.8)	15.6 (14.0–20.0)	12 (0–30)	7.45 (7.31–7.58)	33.4 (25.9–44.4)	18.2 (15.6–20.4)	15 (6–40)
30′ A. O.§	133 (105–156)	7.50 (7.34–7.77)	58.6 (46.1–77.7)	38.1 (32.5–42.6)	26 (0–70)	7.34 (7.21–7.46)	51.1 (43.2–76.2)	17.4 (15.6–21.7)	209 (152–250)
30′ M. V.	113 (100–120)	7.69 (7.55–7.77)	18.6 (14.7–25.4)	20.0 (17.4–25.3)	10 (0–30)	7.56 (7.44–7.73)	28.1 (24.4–40.6)	17.1 (15.4–19.6)	433 (332–530)

* The figures not in parentheses represent the average; the figures in parentheses represent the range.
† Apneic oxygenation.
‡ Mechanical ventilation.
§ Apneic oxygenation with perfusion of 0.3 mM/kg./min. of THAM.

TABLE 2

EFFECTS OF THAM BUFFERED TO pH 7.40 ON HYPERCAPNIC INTRACRANIAL HYPERTENSION IN THREE DOGS

	Arterial Blood				Cerebrospinal fluid				Urine output (ml.)
	Mean pressure (mm. Hg)	pH	$PaCO_2$ (mm. Hg)	HCO_3^- (mEq./l.)	Mean pressure (mm. H_2O)	pH	pCO_2 (mm. Hg)	HCO_3^- (mEq./l.)	
Control	128* (90–150)	7.60 (7.55–7.68)	15.1 (11.0–22.3)	14.2 (10.8–19.1)	36 (25–46)	7.51 (7.45–7.60)	27.1 (20.6–32.7)	20.6 (19.0–21.5)	0
30′ A. O.†	133 (120–150)	6.90 (6.86–6.94)	150.3 (116.7–168.1)	28.6 (24.4–31.6)	153 (70–206)	6.80 (6.73–6.89)	143.6 (131.8–158.9)	21.1 (19.5–24.1)	7 (4–10)
30′ M. V.‡	141 (110–170)	7.60 (7.48–7.67)	19.0 (11.6–30.6)	17.1 (12.2–22.3)	12 (0–20)	7.35 (7.21–7.44)	34.9 (26.4–47.7)	17.3 (16.7–17.9)	15 (12–18)
30′ A. O.§	131 (120–146)	7.00 (6.95–7.04)	116.7 (99.0–148.6)	28.3 (26.2–32.1)	102 (86–110)	6.89 (6.79–7.03)	110.3 (90.1–135.0)	19.8 (17.8–22.3)	180 (150–210)
30′ M. V.	152 (120–176)	7.56 (7.40–7.68)	20.8 (10.6–39.3)	16.1 (12.2–23.8)	−3 (0–10)	7.37 (7.19–7.52)	30.4 (19.9–42.5)	15.7 (15.3–15.6)	453 (390–500)

* The figures not in parentheses represent the average; the figures in parentheses represent the range.

† Apneic oxygenation.

‡ Mechanical ventilation.

§ Apneic oxygenation with perfusion of 0.3 mM/kg./min. of buffered THAM.

age cerebrospinal fluid $p\mathrm{H}$ fell from 7.35 (range 7.21 to 7.44) to 6.89 (range to 7.03), while cerebrospinal fluid $p\mathrm{CO}_2$ rose from 34 mm. Hg (range 26 to o 110 mm. Hg (range 90 to 135), and cerebrospinal fluid HCO_3^- increased 17.2 mEq./l. (range 16.7 to 17.8) to 19.8 mEq./l. (range 17.8 to 22.3). n, urinary output was markedly augmented.

imals treated with D-*mannitol.* Average cerebrospinal fluid pressure LE 3) rose from 30 mm. $\mathrm{H}_2\mathrm{O}$ (range 10 to 40) to 150 mm. $\mathrm{H}_2\mathrm{O}$ (range 116 to after 15 min., and to 155 mm. $\mathrm{H}_2\mathrm{O}$ (range 116 to 188) after 30 min. Mean ial blood pressure rose from 128 mm. Hg (range 96 to 156) to 161 mm. Hg ge 124 to 188). Average arterial blood $p\mathrm{H}$ fell from 7.39 (range 7.29 to) to 6.73 (range 6.59 to 6.83), while PaCO_2 rose from 27 mm. Hg (range 20 5) to 169 mm. Hg (range 141 to 225), and plasma HCO_3^- increased from mEq./l. (range 13.9 to 17.7) to 21.5 mEq./l. (range 20.3 to 23.7). Average orospinal fluid $p\mathrm{H}$ fell from 7.34 (range 7.29 to 7.38) to 6.73 (range 6.57 .85), while cerebrospinal fluid $p\mathrm{CO}_2$ rose from 35 mm. Hg (range 31 to 43) 51 mm. Hg (range 121 to 199), and the cerebrospinal fluid HCO_3^- increased 18.2 mEq./l. (range 17.2 to 19.6) to 18.6 mEq./l. (range 16.7 to 20.5). esis was greatly increased.

nimals treated with urea-invert sugar. Average cerebrospinal fluid pressure LE 4) rose from 33 mm. $\mathrm{H}_2\mathrm{O}$ (range 15 to 50) to 85 mm. $\mathrm{H}_2\mathrm{O}$ (range 65 to after 15 min., and to 100 mm. $\mathrm{H}_2\mathrm{O}$ (range 65 to 135) after 30 min. Mean rial blood pressure rose sharply from 130 mm. Hg (range 120 to 140) to 207 Hg (range 170 to 230). Average arterial blood pH fell from 7.40 (range to 7.53) to 6.64 (range 6.57 to 6.74), while PaCO_2 rose from 22 mm. Hg ge 19 to 25) to 250 mm. Hg (range 212 to 276), and plasma HCO_3^- increased 13.3 mEq./l. (range 11.8 to 15.5) to 26.3 mEq./l. (range 23.4 to 28.4). rage cerebrospinal fluid $p\mathrm{H}$ fell from 7.22 (range 7.17 to 7.29) to 6.74 (range to 6.94), while cerebrospinal fluid $p\mathrm{CO}_2$ rose from 42 mm. Hg (range 33 to o 172 mm. Hg (range 105 to 206), and cerebrospinal fluid HCO_3^- increased 16.0 mEq./l. (range 15.0 to 16.5) to 21.0 mEq./l. (range 20.4 to 21.3). e production was but moderately increased.

Intracranial Hypertension of Acute Brain Compression

ypoventilated dogs. FIGURE 2 shows one example of this type of experiment. owing inflation of the balloon, cerebrospinal fluid pressure rose from 100 $\mathrm{H}_2\mathrm{O}$ to 400 mm. $\mathrm{H}_2\mathrm{O}$. During hypoventilation this pressure increased ner to 940 mm. $\mathrm{H}_2\mathrm{O}$, arterial blood $p\mathrm{H}$ fell from 7.40 to 7.20, and PaCO_2 rose 30 mm. Hg to 133 mm. Hg. A 0.3 M solution of THAM, given intravely at the rate of 1 ml./kg./min. for one hour, corrected both the respiratory osis and the secondary increase in intracranial pressure. This pressure lowered to the level of the pressure in the ballon that was applied at the of the experiment. When the action of THAM subsided and acidosis reed, the cerebrospinal fluid pressure rose again, although an important esis was still present. Arterial blood pressure and cardiac rhythm did not ge significantly throughout the experiment. One dog died shortly after experiment; another one died a few days later from meningeal infection.

ormally ventilated dogs. FIGURE 3 shows one experiment of this type. No

TABLE 3

EFFECTS OF D-MANNITOL ON HYPERCAPNIC INTRACRANIAL HYPERTENSION IN FOUR DOGS

	Arterial blood				Cerebrospinal fluid				Urine output (ml.)
	Mean pressure (mm. Hg)	pH	$PaCO_2$ (mm. Hg)	HCO_3^- (mEq./l.)	Mean pressure (mm. H_2O)	pH	pCO_2 (mm. Hg)	HCO_3^- (mEq./l.)	
Control	122* (104–144)	7.50 (7.40–7.62)	21.5 (11.8–27.0)	15.7 (11.9–17.4)	44 (16–70)	7.47 (7.38–7.54)	29.9 (21.8–35.3)	20.4 (17.7–22.8)	0
30′ A. O.†	150 (128–192)	6.84 (6.78–6.91)	132.5 (98.4–158.1)	21.7 (18.0–23.7)	277 (255–296)	6.87 (6.79–6.94)	125.6 (106.0–167.7)	21.4 (18.0–23.9)	5 (2–10)
30′ M. V.‡	128 (96–156)	7.39 (7.29–7.44)	27.3 (20.9–35.0)	16.1 (13.9–17.7)	30 (10–40)	7.34 (7.29–7.38)	35.6 (31.5–43.2)	18.2 (17.2–19.7)	10 (5–16)
30′ A. O.§	161 (124–188)	6.73 (6.59–6.83)	169.3 (141.7–225.6)	21.5 (20.4–23.7)	155 (116–188)	6.73 (6.57–6.85)	151.2 (121.7–199.8)	18.7 (16.8–20.6)	215 (150–280)
30′ M. V.	145 (100–176)	7.34 (7.10–7.50)	32.5 (20.2–55.9)	16.0 (15.0–16.6)	12 (0–22)	7.25 (7.08–7.37)	40.0 (29.8–55.4)	16.0 (15.1–16.2)	524 (350–640)

* The figures not in parentheses represent the average; the figures in parentheses represent the range.

† Apneic oxygenation.

‡ Mechanical ventilation.

§ Apneic oxygenation with perfusion of 0.6 mM/kg./min. of D-mannitol.

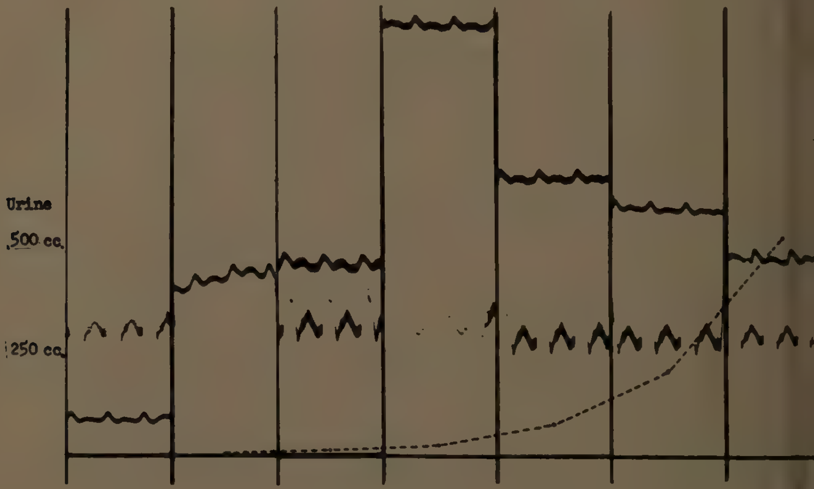
TABLE 4

EFFECTS OF UREA-INVERT SUGAR ON HYPERCAPNIC INTRACRANIAL HYPERTENSION IN THREE DOGS

	Arterial blood				Cerebrospinal fluid				Urine output (ml.)
	Mean pressure (mm. Hg)	pH	$PaCO_2$ (mm. Hg)	HCO_3^- (mEq./l.)	Mean pressure (mm. H_2O)	pH	pCO_2 (mm. Hg)	HCO_3^- (mEq./l.)	
Control	150* (145–155)	7.39 (7.35–7.41)	25.3 (20.0–31.4)	14.9 (12.3–17.6)	53 (40–80)	7.34 (7.21–7.50)	37.6 (27.2–46.2)	18.8 (17.4–20.0)	0
30′ A. O.†	190 (150–210)	6.64 (6.54–6.73)	238.2 (204.3–268.7)	24.9 (22.2–26.4)	247 (175–320)	6.58 (6.32–6.74)	178.2 (148.6–220.0)	18.9 (17.0–21.8)	10 (8–12)
30′ M. V.‡	130 (120–140)	7.40 (7.28–7.53)	21.9 (19.1–25.4)	13.3 (11.8–15.5)	33 (15–50)	7.22 (7.17–7.29)	41.8 (33.1–47.4)	16.0 (15.0–16.6)	13 (10–16)
30′ A. O.§	207 (170–230)	6.64 (6.57–6.74)	250.3 (212.8–276.1)	26.4 (23.5–28.4)	100 (65–135)	6.74 (6.63–6.94)	172.7 (105.7–206.6)	21.0 (20.4–21.4)	72 (15–120)
30′ M. V.	135 (130–145)	7.41 (7.40–7.42)	22.7 (21.5–24.2)	14.0 (13.3–15.3)	8 (0–25)	7.31 (7.13–7.54)	36.2 (23.5–51.9)	16.4 (14.2–18.8)	232 (105–330)

* The figures not in parentheses represent the average; the figures in parentheses represent the range.
† Apneic oxygenation.
‡ Mechanical ventilation.
§ Apneic oxygenation with perfusion of 30 per cent urea in 10 per cent invert sugar at the rate of 3 ml./min.

fall in ventilation took place and the blood acid-base balance remained wit physiological limits. Inflating the balloon resulted in a rise of the cerebrospi fluid pressure from 130 mm. H_2O to 540 mm. H_2O and, subsequently, the p tern was that of a plateau. After 30 min. had elapsed, 30 per cent urea in per cent invert sugar was given intravenously at the rate of 3 ml./min. for hour. Intracranial pressure decreased to 38 mm. H_2O, well below the cont value. Arterial blood pressure increased slightly toward the end of the exp ment, and brachycardia occurred. All dogs in this group survived.



Time	0		30m	90m	120m	150m	190m
ABpH	7.48		7.50	7.20			7.82
$paCO_2$	30.5		29.0	133.6			58.6
HCO_3^-	21.8		22.0	63.8			92.6
CSFP (mm.H_2O)	100	400	445	940	635	600	420

B.I. + M.V.

← THAM 0.3mM/kg/min., 60 min. —

FIGURE 2. Acutely induced intracranial hypertension: correction with 2-amino-2- droxymethyl-1,3-propanediol.

DISCUSSION

The first series of experiments confirm the effectiveness of THAM in c recting the blood acid-base disturbances and intracranial hypertension indu by hypercapnic acidosis as reported previously.[8,10] That THAM a through both its osmotic and buffering properties may be seen from the f that, when buffered THAM is administered during apneic oxygenation the in cerebrospinal fluid pressure is only partially corrected.[11] Results similar those observed with buffered THAM occur when 0.6 *M* D-mannitol is ministered in an amount which will produce a diuresis similar to that of THA but will not correct acidosis. Even a very hypertonic solution such as ur

ose (5.8 *M*) did not completely correct the intracranial hypertension of
capnic acidosis. The partial correction of hypercapnic intracranial hyper-
on by agents exerting an osmotic activity cannot be explained on the bases
present findings. Such correction could indicate that hypercapnic acido-
d its accompanying cerebral vasodilatation may also be accompanied by
fer of water from vascular to cerebrospinal compartments, or possibly from
to intracellular spaces. This hypothesis requires further experimental

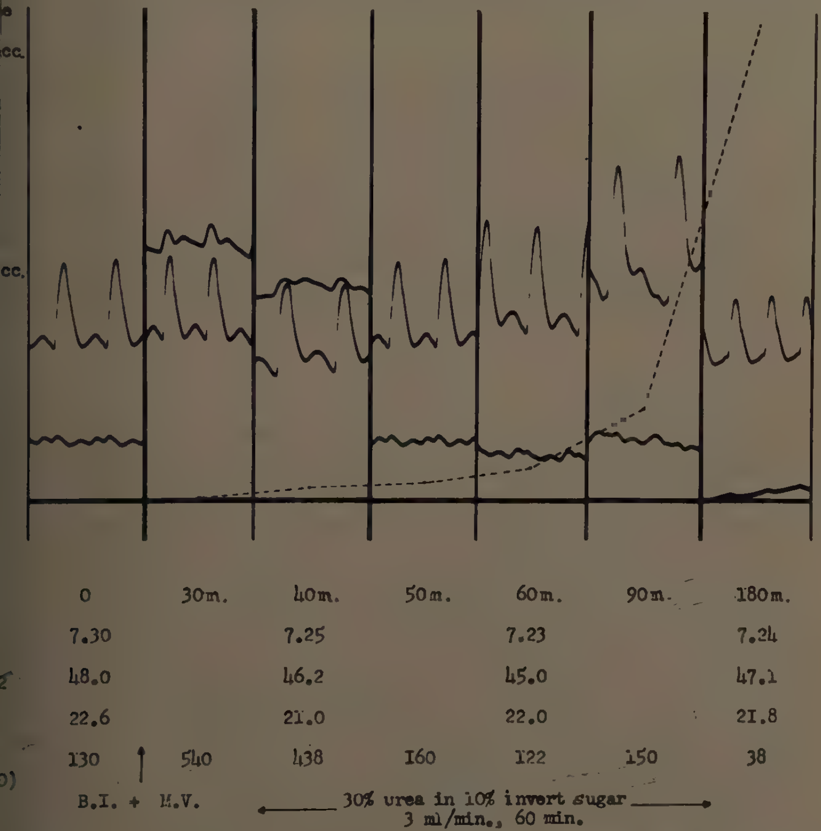


FIGURE 3. Acutely induced intracranial hypertension: correction with urea.

ng. Intracranial hypertension of hypercapnic acidosis can be corrected
by hyperventilation[12] or by administration of THAM. Relationships be-
increase in cerebrospinal fluid pressure and acid-base changes in blood and
rospinal fluid cannot be discussed on the basis of the present measure-
s. In these experiments, samples of cerebrospinal fluid were replaced by
, a procedure that altered the bicarbonate concentration and acid-base
ce therein.

e importance of hypercapnic acidosis in the genesis of intracranial hyper-
on was further confirmed in the second series of experiments when severe
ression of the brain was accompanied by hypoventilation and a secondary

increase in intracranial pressure. In these experiments, intracranial press increased again when THAM administration was stopped and while signific diuresis was still present. This fact indicates that the most important fac in the correction of intracranial hypertension of acute brain compression is increase in blood osmolality. As shown by Javid,[13] such a correction is bet maintained in the absence of urinary excretion. As THAM is a potent diure its action to relieve the intracranial hypertension associated with brain co pression may be limited.

CONCLUSION

Intracranial hypertension of hypercapnic acidosis cannot be corrected agents such as a 5.8 M solution of urea and invert sugar, which only ha osmotic properties. Such a correction requires the restoration of the acid-b balance to normal, either by administration of THAM or by hyperventilati

Intracranial hypertension induced by acute brain compression may prod hypoventilation and hypercapnic acidosis which, in turn, will induce a seco ary rise in intracranial pressure. The best correction of intracranial press may be performed by a solution that would maintain acid-base balance const and also increase the osmolality of the blood.

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V. Amine Buffers as Pharmacological Agents

HE HYPOGLYCEMIC ACTIVITY OF 2-AMINO-2-HYDROXY-METHYL-1,3-PROPANEDIOL*

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r observation that intravenous administration of 2-amino-2-hydroxy- yl-1,3-propanediol, Tris or Tham, could bring about severe hypoglycemia rmal humans and dogs[1] deserved further elucidation in view of the proposed al uses for this compound. Interest in the means of controlling or pre- ing this effect when it is not desired, or of taking advantage of it, is am- d by the anticipation of gaining some theoretic knowledge concerning se metabolism. Further, the widespread use of Tris in cell and tissue a not only demands knowledge of these effects in intact animals, but also us the advantage of later integrating our findings with cellular events. e present here our findings in intact and pancreatectomized dogs, especially ey relate to insulin action.

Methods

ongrel dogs weighing between 15 and 20 kg., maintained on a constant were fasted for 18 to 20 hours before intravenous infusion of 0.3 M or 0.6 M . They were restrained in a canvas jacket. Venous sampling was an indwelling cannula kept patent with heparin saline and thoroughly d with outflowing blood before the samples were taken. Infusion rates constantly monitored. All blood samples were centrifuged immediately protein-free filtrates made within a few minutes. Plasma sugar was deter- d by the Nelson-Somogyi method.[2] Glucose was simultaneously deter- d in representative series of plasmas by the enzymatic glucose oxidase od.[3] Plasma inorganic phosphate was determined by the method of e and Subbarow;[4] sodium and potassium by internal standard flame ometry (Barclay); chloride by Volhard titration, and hemoglobin by the rption of cyanmethemoglobin at 540 mμ. Whole blood pH was read immediately on a Cambridge Model R meter equipped with a jacketed glass rode maintained at 38° C. with circulating oil. All our methods were ked for interference by Tris. It was found to cause a slight effect on flame ometric determination of sodium.

ancreatectomized dogs were maintained postoperatively by the method of kowitz.[5] The dogs were maintained on crystalline insulin and a constant supplemented with pancreatic extract† and vitamins. All have remained od health with no weight loss after initial recovery.

irect perfusion of the pancreas of anesthetized dogs was accomplished ugh the pancreatic artery, entry being made as high as possible. The

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Pancreatin, Eli Lilly and Company.

perfused area was checked initially by blanching with saline and termin with methylene blue. In two cases a second cannula was introduced i lower portion of the pancreatic artery in addition, since collateral circulat prevents the spread of perfusing fluid to this area. Nembutal was used anesthesia. All experimental groups consisted of 3 or more dogs—usually

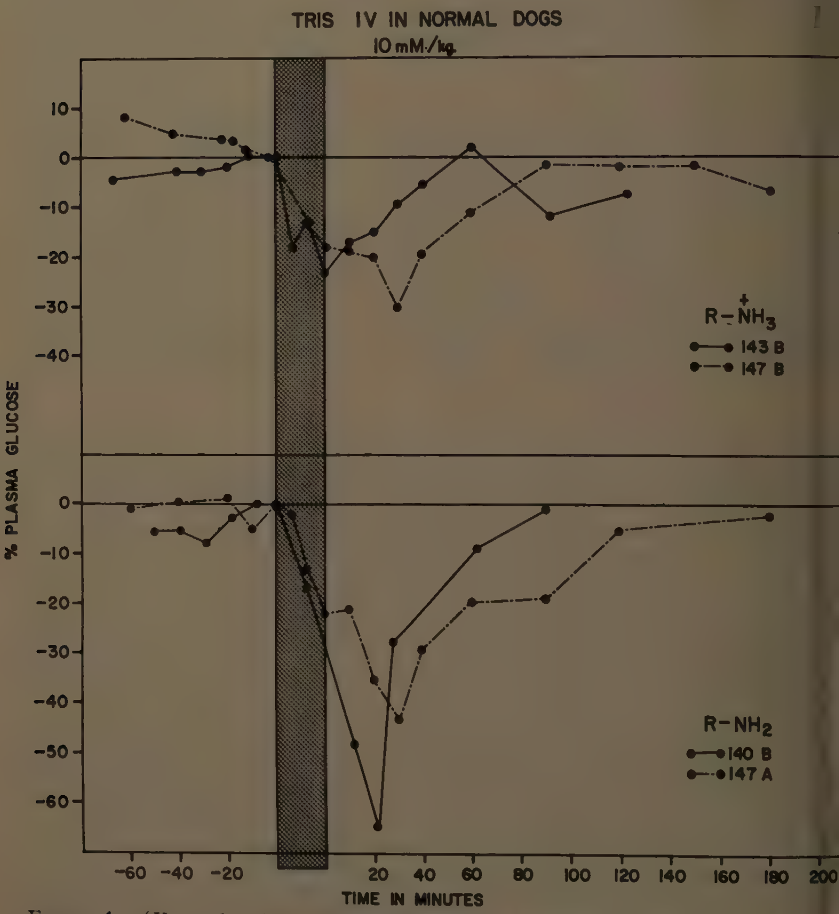
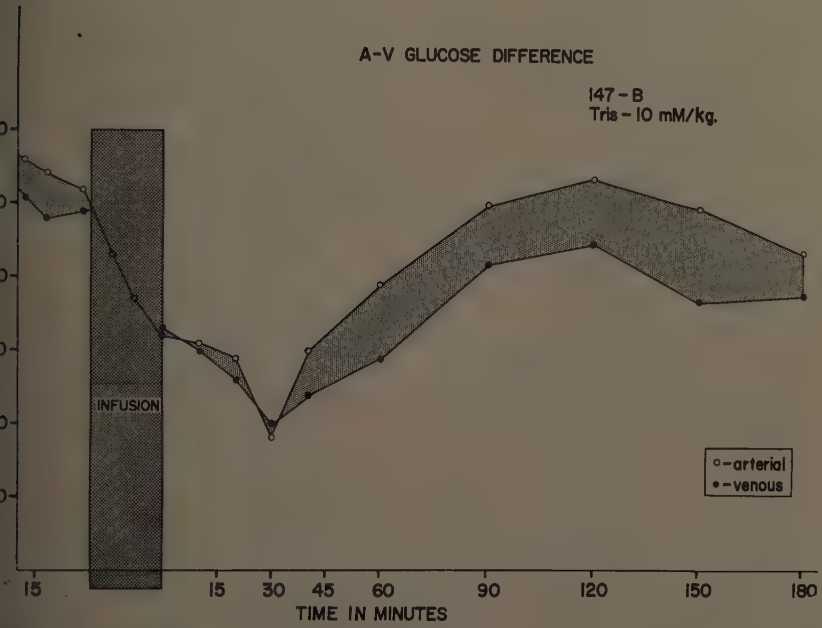


FIGURE 1. (*Upper figure*) Series B: effect on plasma glucose in 2 normal dogs of 10 mm kg. 2-amino-2-hydroxymethyl-1,3-propanediol titrated to *p*H 6.1 ($R-NH_3$) and given 17 mins. (*Lower figure*) Series A: Effect on plasma glucose of the same quantity of Tris the same time but with *p*H not adjusted, that is, Tris in the form of $R-NH_2$.

Results

Series A: normal dogs receiving alkaline Tris ($R-NH_2$). Following intraven infusion of 10 to 12 mmole/kg. of Tris (*p*H 10.2) over 17 min. to norr awake dogs there was a reduction of plasma glucose of 30 to 50 per cent (F URE 1) maximal 20 min. after the end of infusion and returning to near c trol values after 1 to 2 hours. Plasma inorganic phosphate began to d immediately upon infusion and continued to fall precipitously for 40 to 60 m only started to recover at the end of 2 hours. With these rapid infusions na potassium rose 20 per cent during infusion but then declined very rap- to preinfusion levels. Whole blood pH rose 0.21 to 0.28 pH U., but dropped rapidly usually to 7.50 when glucose was at its lowest. In only 1 of 4 nor- dogs has there been any significant drop (13 per cent) in plasma glucose wing the administration of 4 mmole of Tris/kg. One dog that was un- ted had received 12 mmole/kg. of sodium bicarbonate before the Tris. mal controls that received 0.3 M sodium bicarbonate (8 mmole/kg. to pH ; 10 mmole to pH 7.57, 12 mmole to pH 7.64) showed no fall in plasma ose.



URE 2. Arterial and venous plasma glucose following administration of 10 mmole/kg. s I.V. at pH 6.1 to a normal dog.

ries B: normal dogs receiving acid Tris (*R-NH*$_3$). Following administra- of 10 to 14 mmole/kg. of Tris exactly as in Series A except that the Tris acidified to pH 6.1, there was a 21 per cent reduction in glucose at the end usion (FIGURE 1); recovery was within 20 min. in all but one dog; phos- e continued to fall for 2 hours as with alkaline Tris. FIGURE 2 shows iovenous differences in one of these dogs. Eight mmole of Tris/kg. ed to an intermediate pH of 7.1 gave the same effect as 12 mmole at pH A single dog that received 27 mmole/kg. Tris at pH 7.4 over an hour mmole/kg. min.) showed a gradual drop in blood sugar starting at 25 and continuing to 45 mg. per cent (−60 per cent) at the end of infusion. very occurred at the same rate as the fall over a 1-hour period; phosphate ined at less than 1 mg. per cent; CO$_2$ fell slightly; pH fell to 7.23.

ies C: diabetic dogs receiving alkaline Tris. Alkaline Tris (0.3 M or 0.6 M,).2 or 10.6) given in doses of 4, 5, 7, 8, and 10 mmole kg. over 17 min. has never given any significant reduction in plasma glucose in 4 pancreatectomiz (diabetic) dogs deprived of food and insulin for 18 to 24 hours. Twelve mmol kg. of 0.3 M Tris caused a transient mean drop of 12 per cent in 5 infusions the sample taken during the first 10-min. interval but not thereafter. Wh 0.6 M or 0.9 M Tris was used even this transient drop did not occur. Plasm

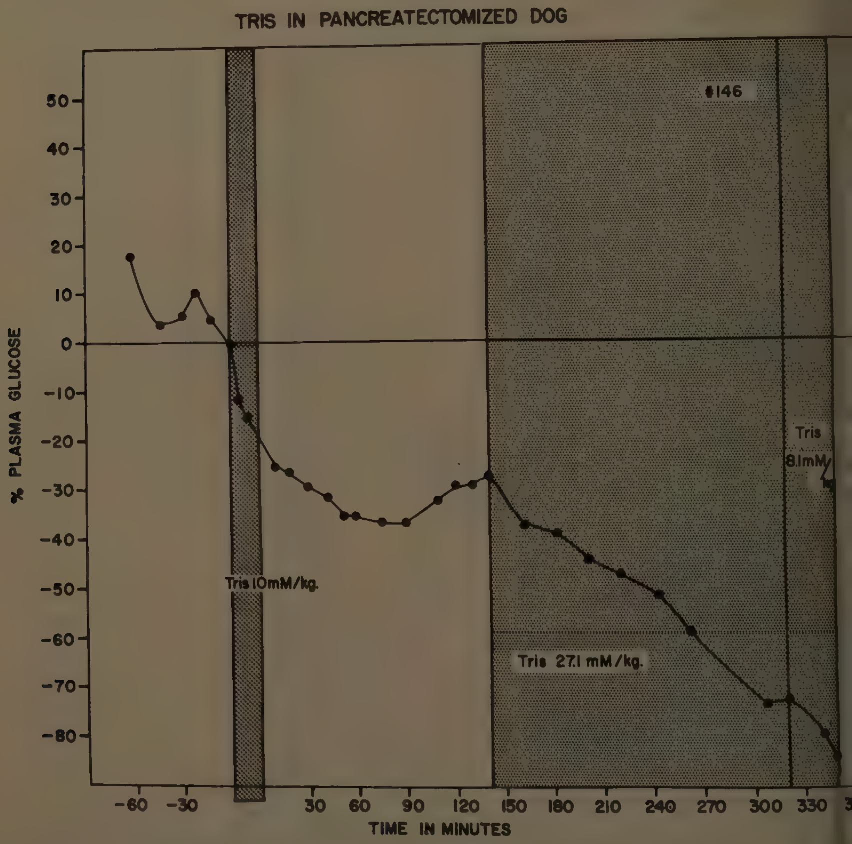


FIGURE 3. Typical plasma glucose results following administration of Tris I.V. to anaesthetized dog 2 hours following pancreatectomy. Infusion I: 0.6 mmole/kg./mi infusion II: 15 mmole/kg./min.; and infusion III: 0.3 mmole/kg./min.

potassium was frequently raised as much as 55 per cent; inorganic phospha was usually lowered 50 to 60% per cent; pH was raised 0.25 pH U.

Three groups of control studies were done in these diabetic dogs: (1) infusi of 12 mmole/kg. of 0.3 M sodium bicarbonate caused falls of 10 to 25 per cent plasma glucose that persisted 2 hours; (2) saline caused a consistent 20 per ce lowering that also lasted 2 hours; (3) even following the sham procedure of straining the diabetics, declines of 2 to 14 per cent were observed.

If, however, one infuses 10 mmole of Tris over 17 min. into a dog withi few hours after pancreatectomy, hypoglycemic responses comparable to th in normal dogs occur. FIGURE 3 shows a typical response. There is lit

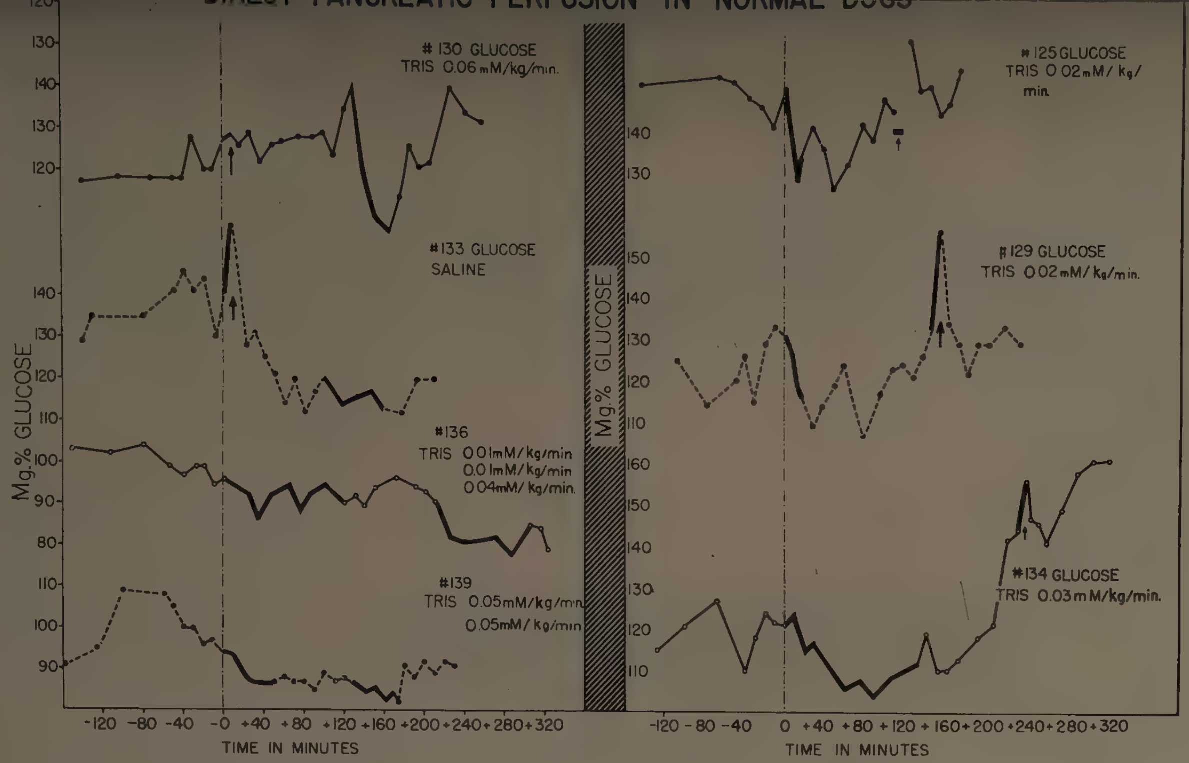


FIGURE 4. Series D: plasma glucose values following the administration of Tris or glucose directly into the pancreatic artery in normal dogs. Arrows indicate injection of glucose; heavy lines without arrows designate perfusion with the Tris solutions indicated.

tendency to recover after 2 hours. Controls show rises, as would be expecte throughout a comparable period. If the infusion is carried further one can driv the glucose down to 30 mg. per cent at death (35 mmole/kg. total dose in th case).

Series D: Direct perfusions of the pancreas of 9 normal anesthetized do were performed. Tris adjusted to *p*H 7.4 to prevent tissue damage, an delivered at rates from 0.02 to 0.06 mmole/kg. brought about a lowering plasma glucose of about 10 mg. per cent, which was maintained over 1½ to hours. This lowering was equal to, or greater than, that following injection

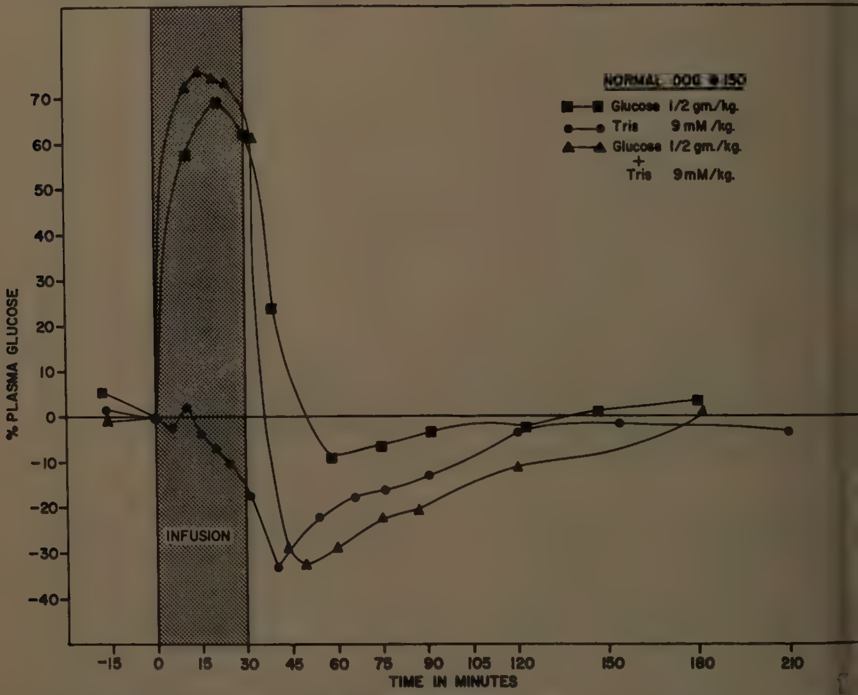


FIGURE 5. Comparison of the effect on plasma glucose of intravenous Tris alone a Tris given with glucose to that following the administration of glucose alone (normal gluc tolerance curve).

5 per cent glucose (FIGURE 4). Results of perfusions were not considered initial plasma glucose level was above 125 mg. per cent.

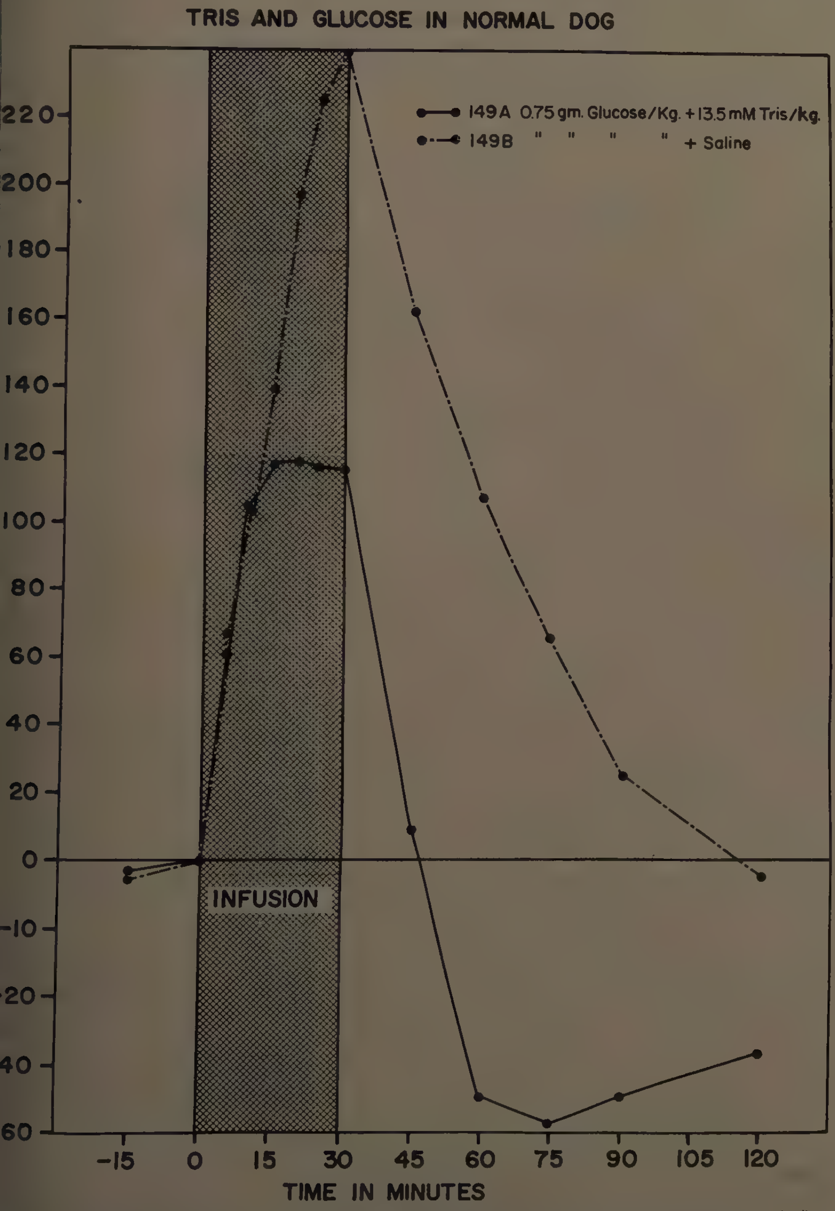
Series E: Recently we have administered Tris (9 mmole/kg.) and/or gluc (0.5 gm./kg./hour) in the manner of a glucose tolerance test over a half-hour p riod in order to observe relative timing of the Tris hypoglycemic reacti From these results (now 7 pairs of infusions) the following points are emergin

(1) Tris hypoglycemia, like the hypoglycemic response to glucose, becom apparent in about 20 min., has a maximum at 40 min., and is compensat within 2 hours. These time relations do not shift (FIGURE 5).

(2) Tris hypoglycemia is not preceded by hyperglycemia.

(3) The administration of potassium with Tris increases the severity of t hypoglycemia. The time relations are not changed.

4) Tris does not reduce the hyperglycemia caused by glucose administered avenously at a rate reported to be near maximal intestinal absorption rate gm./kg./hour)[6] but if more glucose is given, so that one does not have a mal glucose tolerance curve, Tris will reduce this hyperglycemia as shown in URE 6.



IGURE 6. Plasma glucose curve following the I.V. administration of 13.5 mmole Tris/kg. 0.75 gm./kg. glucose (1½ × maximal intestinal absorption rate) compared with that wing administration of the same quantity of glucose alone.

(5) The magnitude of reduction of plasma glucose by Tris is the same whe
additional glucose is administered with it.

(6) A second challenge with Tris and sugar, immediately upon recovery from
first, results in a hypoglycemia of the same magnitude and duration as th
first.

(7) A second dose of 9 mmole/kg. of Tris given within 45 min. of the first, an
before recovery, prolongs the recovery period but does not increase the magn
tude of the hypoglycemia.

Discussion

It is apparent that the hypoglycemia following Tris administration is a high
predictable response in a normal dog, dependent upon the two sliding facto
of total dose and rate of administration. The recovery from a single rapid
administered dose is also predictable. However, recovery from a dose admi
istered more slowly over a long period of time is less clear. We have show
that in the rat liver glycogen is very rapidly depleted by Tris administration

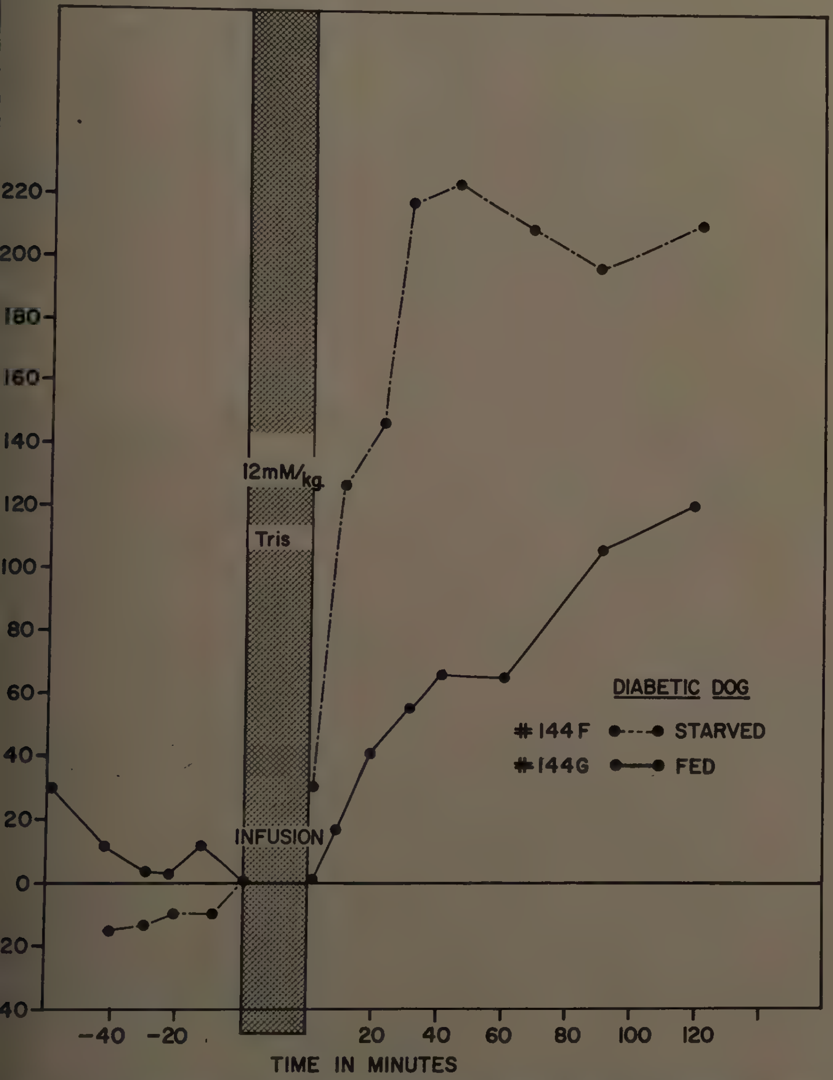
Therefore the failure to recover after long infusion times may be a co
sequence of depleted liver stores. We have eliminated in our control studi
the possibility that this reaction is due to hemodilution or to the raising of blo
*p*H. Furthermore the time characteristics of this reaction follow close
those of insulin stimulation. The time of onset, time of maximum rate of fa
minimum point, and point of recovery following a discontinuous dose of Tr
are identical to those following glucose administration.

Other characteristics of insulin stimulation are also apparent, as shown
FIGURE 2. Arteriovenous differences in blood glucose are increased as they a
following insulin.[8] The response is not ordinarily elicited in alloxan diabet
rats. Adrenalectomized rats show increased sensitivity to it. Convulsio
may be produced which are relieved by the injection of glucose.[7] Howeve
each of these points may be called upon merely to describe events followi
insulin stimulation rather than to prove that such has occurred. We ca
not bridge this gap between cause and effect. We know that there is i
creased glucose uptake, but one must have more direct evidence to deci
whether it was brought about by increased insulin stimulation.

We have attempted to determine whether Tris perfused directly into t
pancreas in quantities that do not ordinarily produce hypoglycemia if a
ministered peripherally will cause lowering of blood sugar. We have alwa
found a lowering of the same magnitude as that following glucose administ
tion directly to the pancreas. We have never seen hypoglycemia of t
magnitude obtained with peripheral administration of any of the doses
scribed here. We are unable to deliver alkaline Tris directly to the pancre
because of resultant tissue damage. These perfusions have all been carried
with Tris adjusted to *p*H 7.4. Peripherally administered Tris at *p*H 7.4 h
been shown to be active.

Although it seems likely that stimulation of insulin secretion follows T
administration, is this its only or even major effect on glucose metabolis
Certainly the diabetic dog off insulin for 18 or more hours is recalcitrant to T
administration. The small drops seen by Dos[9] certainly can be due to ot

ors as shown in our control diabetic studies. However, we have shown
t there is no need for the pancreas to be present, that is, for insulin to be
ulated, to demonstrate the hypoglycemic reaction to Tris. Dogs pan-
tectomized for only a few hours show it, and the fed diabetic appears to be
t under some control by it for a time after plasma glucose has begun to
nb, 6 hours after insulin administration. Starved diabetics were not so
trolled by Tris (FIGURE 7).



IGURE 7. A comparison of the change in plasma glucose following intravenous Tris ad-
stration to a fed diabetic (pancreatectomized) dog on insulin with that when the same
vas starved for 69 hours. Plasma glucose value at time of infusion was 80 mg. per cent
th cases.

We suggest, therefore, that a second effect of Tris on plasma glucose i facilitation of glucose uptake *when insulin is acting* rather than a result o quantitatively greater stimulation of insulin per se. There are other reas for thinking this: (1) the relatively large quantity of Tris necessary to ca hypoglycemia; (2) the necessity for maintaining its concentration in order maintain its effect on blood sugar; (3) Tris can facilitate lowering of a *very h* blood sugar when it does not lower the peak of glucose tolerance curv (4) fed animals seem to be more sensitive to Tris hypoglycemia than star ones; and (5) administration of potassium with Tris augments its hypog cemic effect.

In order to come closer to an understanding of the hypoglycemic activity Tris we are now attempting to clarify its relation to adrenal function and relation to the reduction in oxygen saturation which large doses may produ

It is interesting to note in this regard that Randle and Smith[10] used Tris *vitro* in determining the effects of anaerobiosis on glucose uptake of isolated diaphragm, and found that in Tris medium, in contrast to bicarbonate mediu glucose uptake was not stimulated under anaerobic conditions. He conclu that this was due to a fall in *p*H in the Tris medium which did not occur bicarbonate. Clearly we have no parallel with this in the intact animal.

Summary

Tris in doses of 10 and 12 mmole/kg. in single rapid intravenous infusi into normal dogs caused a lowering of blood sugar of 40 to 50 per cent wit 20 min. after infusion. Blood glucose then rose gradually over one hour turning to normal by 1½ to 2 hours.

Hypoglycemia following Tris adjusted to *p*H 6.1 was less severe and recov was more rapid.

Pancreatectomized diabetic dogs off insulin and food for 18 to 24 hours not show any significant reduction in blood glucose with administration of T

Blood glucose was lowered in pancreatectomized dogs receiving Tris withi few hours after pancreatectomy.

Direct pancreatic perfusion suggested that Tris could stimulate ins secretion.

When 9 mmoles of Tris were administered intravenously to normal d over one-half hour the consequent hypoglycemia occurred at the same tim that following glucose administration.

Administration of Tris with glucose did not lower the hyperglycemic peak increased the subsequent fall in the glucose tolerance curve.

We suggest that the direct effect of Tris upon glucose uptake is sufficien have serious consequences if the carbohydrate stores of an animal are depl and/or if administration is continued over a long period of time.

Finally, we suggest that Tris facilitates the activity of insulin.

Acknowledgments

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EFFECTS OF 2-AMINO-2-HYDROXYMETHYL-1,3-PROPANEDIOL ON SYSTEMIC VASCULAR RESISTANCE AND REACTIVITY DURING ENDOTOXIN SHOCK

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Peripheral vascular collapse has been observed both in humans and in d
following the penetration of the blood stream by bacteria.[1,2] The organis
most frequently encountered in Bacteremia are those of the coliform gro
that characteristically release endotoxin.[3] The response of the body to the
endotoxins is varied, but usually consists of a hypotensive episode, an arter
blood acidosis, oliguria, and hemoconcentration.[4]

Therapeutic measures utilized to combat these changes are numerous, b
in character and in their ultimate effect on the subject. Among the age
commonly employed are antihypotensives such as Metaraminol and nore
nephrine, adrenal cortical steroids, and large infusions of plasma. Previous w
from this laboratory has indicated the relative effectiveness of these agents
At best a combination of the various agents has been able to ameliorate t
hypotensive episode of endotoxin shock while an unaltered course of re
failure and metabolic acidosis commonly leads to death.

In an effort to evaluate THAM* as an agent that might increase the outp
of urine and relieve the acidosis without decreasing blood pressure, the followi
study was undertaken.

Methods

A lipopolysaccharide suspension of *Escherichia coli* endotoxin was u
throughout all experiments in a dosage so standardized that a single intraven
injection (0.55 mg./kg.) resulted in death within 24 hours.

Adult male mongrel dogs were anesthetized with 30 mg./kg. sodium pen
barbital. Endotoxin was injected into the femoral vein of each animal a
observations were then made continuously from 8 to 10 hours. Syste
arterial pressure was measured by a catheter placed in the femoral artery a
recorded on a Sanborn Twin Viso. An indwelling polyethylene catheter
inserted into the urinary bladder and the urine was collected hourly. The
and hematocrit of the arterial blood were measured hourly.

The study was carried out on 48 dogs divided into 5 groups. The f
group, comprised of 12 dogs, acted as controls in that they received endoto
alone. The second group, of 6 dogs, was treated with THAM at a time w
the animals showed oliguria, acidosis, and hypotension. A third group, of
dogs, was treated with THAM and Metaraminol, while a fourth group, of
dogs, received hydrocortisone and THAM. The fifth group, of 10 dogs,
treated with a combination of THAM and hydrocortisone followed by Meta
minol.

In all groups, dogs were considered permanent survivors if they resu
normal physical activity within 48 hours postendotoxin.

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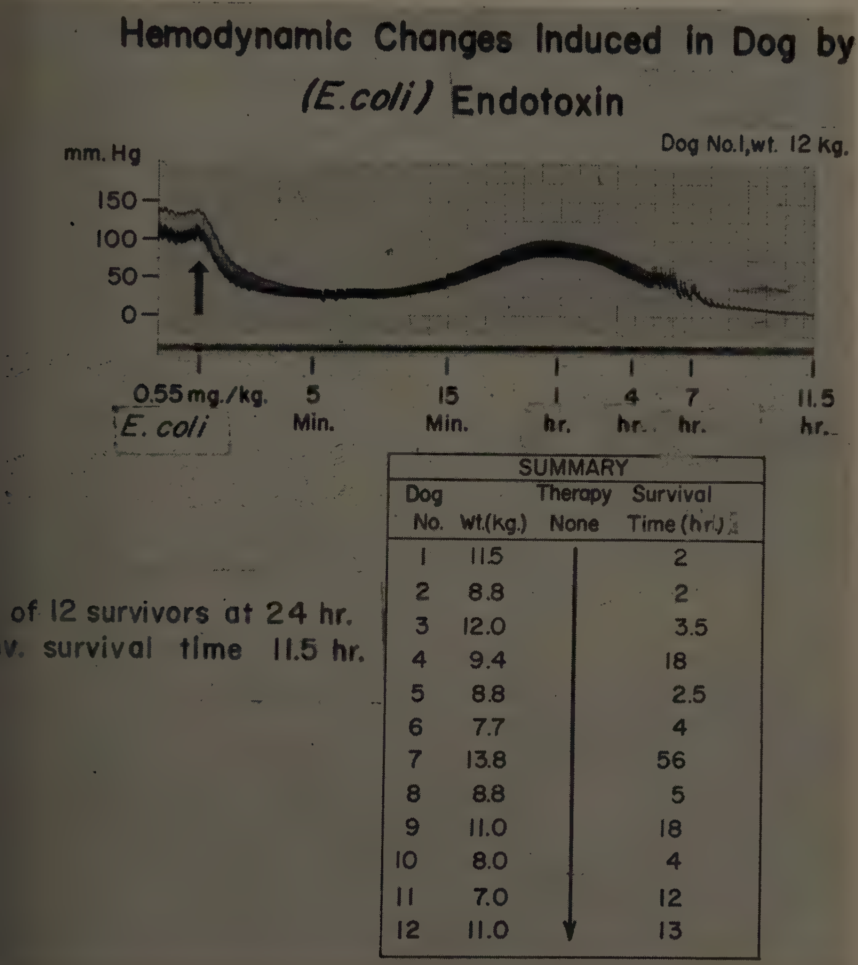
* 2-Amino-2-hydroxymethyl-1,3-propanediol.

Results

The average time of survival of the 12 control dogs was 11.5 hours, although dog survived for 56 hours. These control dogs showed the characteristic



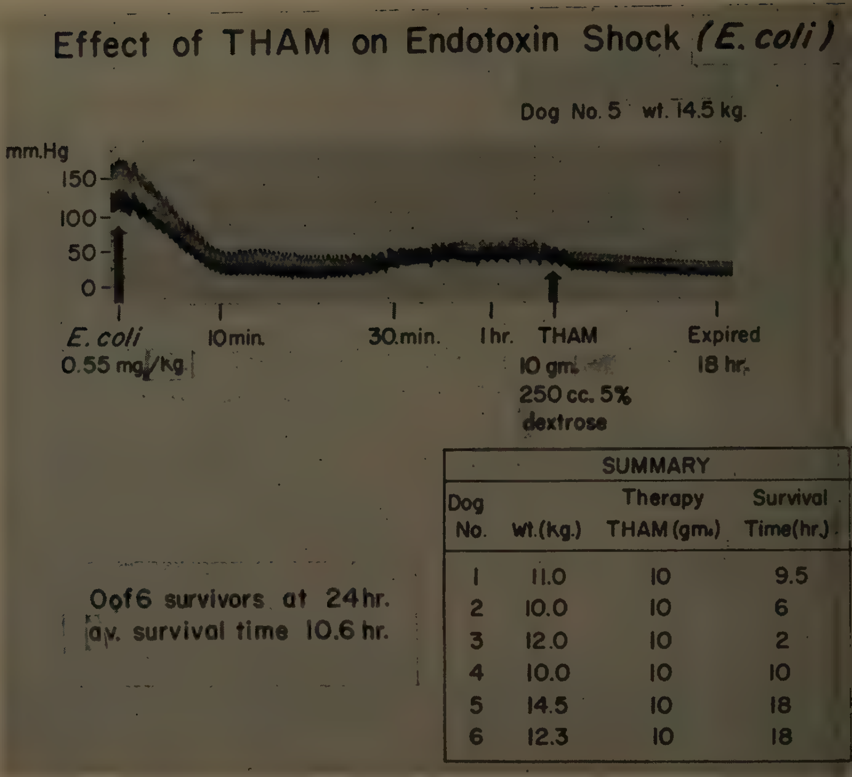
of 12 survivors at 24 hr.
av. survival time 11.5 hr.

SUMMARY

Dog No.	Wt.(kg.)	Therapy None	Survival Time (hr.)
1	11.5	↓	2
2	8.8		2
3	12.0		3.5
4	9.4		18
5	8.8		2.5
6	7.7		4
7	13.8		56
8	8.8		5
9	11.0		18
10	8.0		4
11	7.0		12
12	11.0		13

FIGURE 1. Hemodynamic changes induced in dogs by *E. coli* endotoxin.

itial hypotension followed by a recovery phase and, finally, the development irreversible shock starting approximately 2 hours postendotoxin. FIGURE 1 ows the average data on this group with a pressure tracing from a representave animal. The average hematocrit rose from 39 before endotoxin to 69 at hours postendotoxin. During this time the average *p*H dropped from 7.40 7.24. Renal failure was a common terminal event, the urine flow showing a op from a control level of 7.2 cc./hour to 0.6 cc./hour at 9 hours.



Dog No.	Wt. (kg.)	Therapy THAM (gm.)	Survival Time (hr.)
1	11.0	10	9.5
2	10.0	10	6
3	12.0	10	2
4	10.0	10	10
5	14.5	10	18
6	12.3	10	18

FIGURE 2. Effect of THAM on endotoxin shock (*E. coli*).

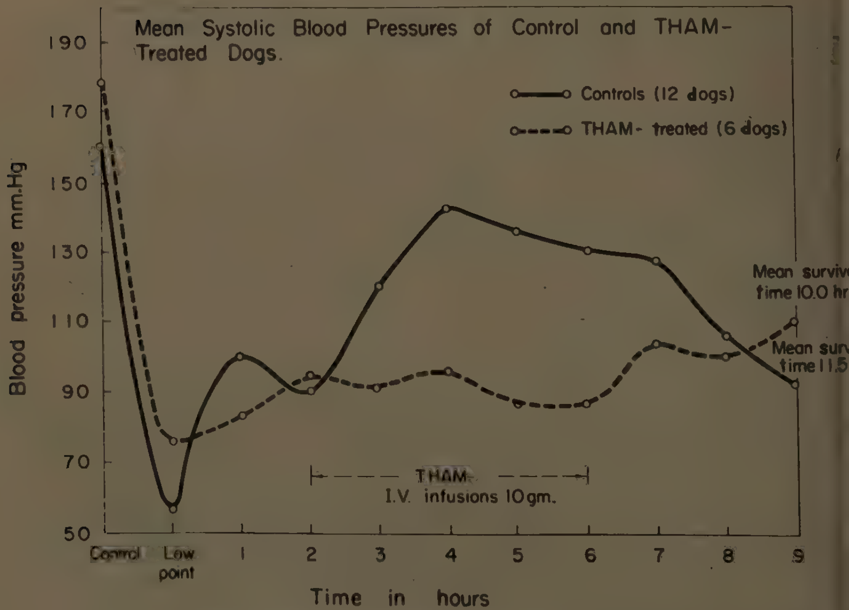
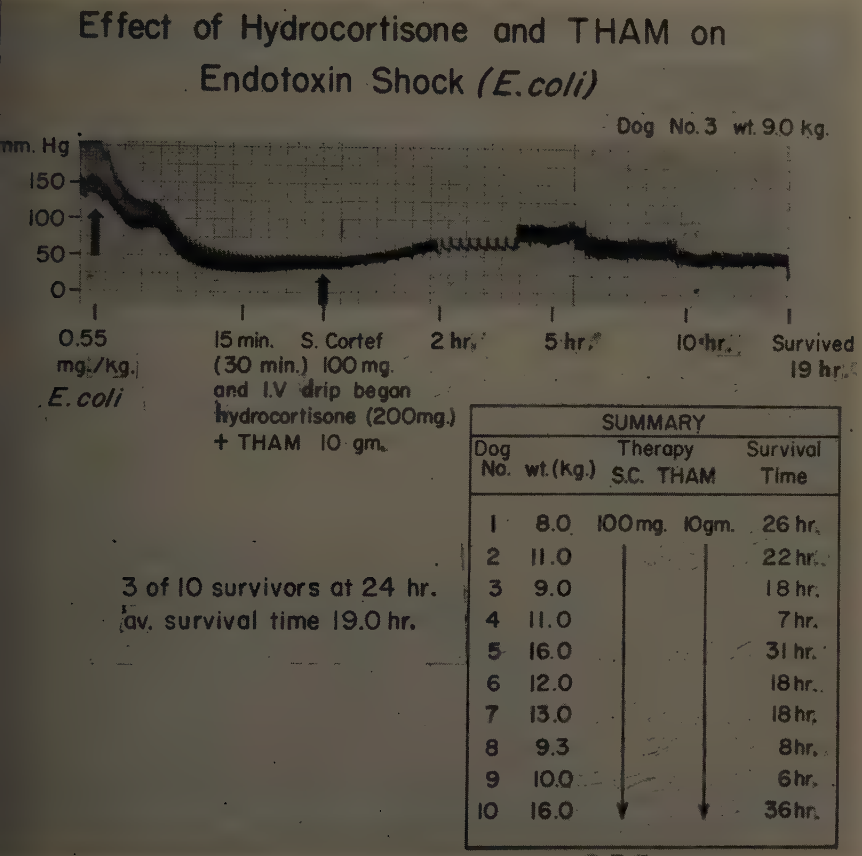


FIGURE 3. Mean systolic blood pressures of control and THAM-treated dogs.

The survival time of the 6 dogs treated with 10 gm. of THAM averaged 10.6 ours (FIGURE 2). There were no 24-hour survivals. This group showed a ean hematocrit rise from 45 to 54 in 5 hours, with a drop to 50 at 10 hours. here was an increase in mean blood pH from 7.50 to 7.60 at 9 hours, while rine output increased from a control of 16 cc./hour to a high of 35 cc./hour, nd dropped to 12 cc./hour at the end of the observation period.



SUMMARY

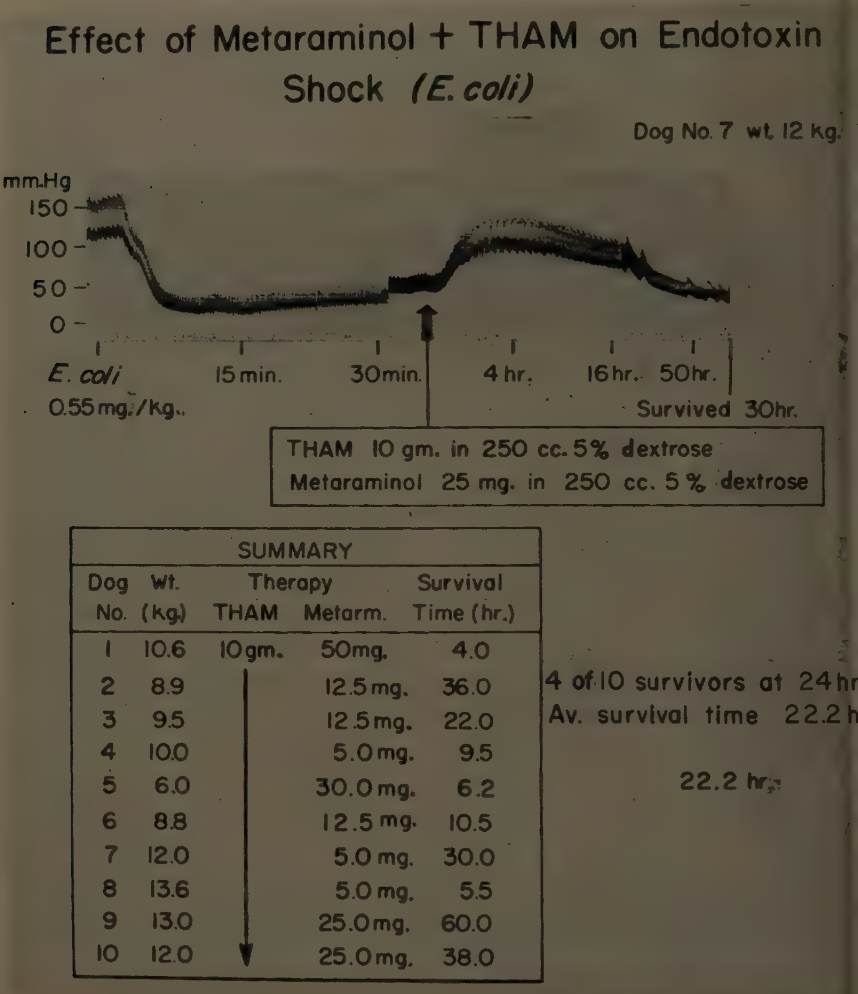
Dog No.	wt. (kg.)	Therapy S.C.	Therapy THAM	Survival Time
1	8.0	100 mg.	10 gm.	26 hr.
2	11.0			22 hr.
3	9.0			18 hr.
4	11.0			7 hr.
5	16.0			31 hr.
6	12.0			18 hr.
7	13.0			18 hr.
8	9.3			8 hr.
9	10.0			6 hr.
10	16.0			36 hr.

FIGURE 4. Effect of hydrocortisone and THAM on endotoxin shock (*E. coli*).

FIGURE 3 shows the pressure time course of the control and THAM-treated imals. THAM was infused for a period of 2 to 6 hours postendotoxin.

The average survival time of the 10 dogs treated with hydrocortisone and HAM was 19.0 hours. Although 3 of the dogs remained alive at 24 hours, ere were no permanent survivors. Data from this group are given in FIGURE

The results in the 10 animals treated with Metaraminol and THAM are ven in FIGURE 5. The average survival time was increased to 22.2 hours with 4 of 10 survivors at 24 hours. As in the previous group, there were r permanent survivors. The blood pressure effect of Metaraminol on these dog is characterized by the tracing in FIGURE 5.



SUMMARY

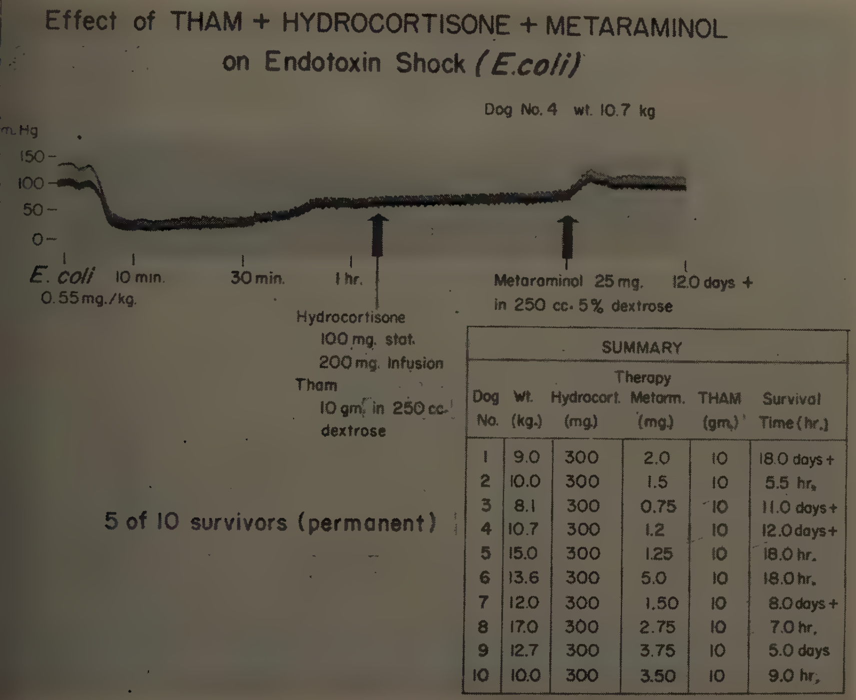
Dog No.	Wt. (kg.)	Therapy THAM	Therapy Metarm.	Survival Time (hr.)
1	10.6	10 gm.	50 mg.	4.0
2	8.9	↓	12.5 mg.	36.0
3	9.5		12.5 mg.	22.0
4	10.0		5.0 mg.	9.5
5	6.0		30.0 mg.	6.2
6	8.8		12.5 mg.	10.5
7	12.0		5.0 mg.	30.0
8	13.6		5.0 mg.	5.5
9	13.0		25.0 mg.	60.0
10	12.0		25.0 mg.	38.0

FIGURE 5. Effect of Metaraminol + THAM on endotoxin shock (*E. coli*).

The treatment of 10 dogs with a combination of THAM, hydrocortisone, a Metaraminol therapy resulted in 5 of 10 permanent survivors. FIGURE presents the data from this group along with a representative blood pressu tracing.

A more successful therapy was seen, however, in a group of 10 dogs treat with Metaraminol and hydrocortisone (FIGURE 7).

FIGURE 8 shows a comparison of the pressure changes in this last-mentioned roup and another group treated with Metaraminol, THAM, and hydrocor-sone. The animals not treated with THAM showed 6 of 10 permanent sur-ivors. The average time for the initiation of THAM therapy in this group as 2 hours postendotoxin; therapy was maintained as a slow I.V. drip for ap-roximately 4 hours.



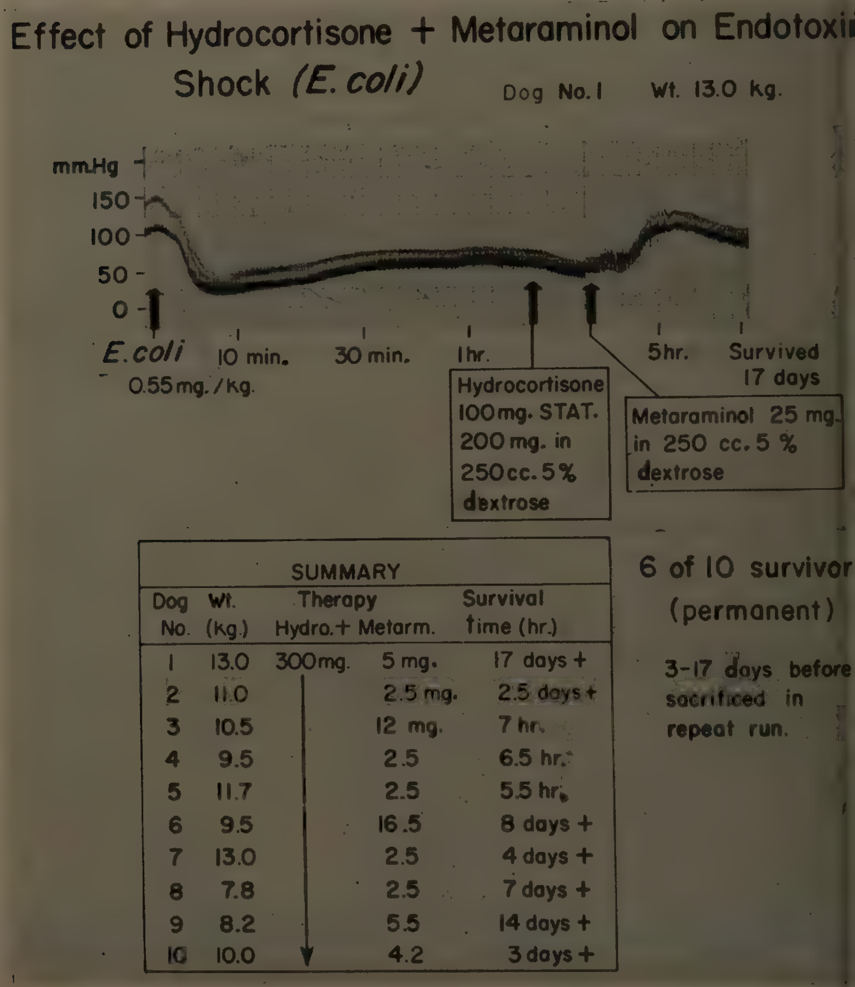
SUMMARY

Dog No.	Wt. (kg.)	Therapy Hydrocort. (mg.)	Therapy Metarm. (mg.)	Therapy THAM (gm.)	Survival Time (hr.)
1	9.0	300	2.0	10	18.0 days +
2	10.0	300	1.5	10	5.5 hr.
3	8.1	300	0.75	10	11.0 days +
4	10.7	300	1.2	10	12.0 days +
5	15.0	300	1.25	10	18.0 hr.
6	13.6	300	5.0	10	18.0 hr.
7	12.0	300	1.50	10	8.0 days +
8	17.0	300	2.75	10	7.0 hr.
9	12.7	300	3.75	10	5.0 days
10	10.0	300	3.50	10	9.0 hr.

FIGURE 6. Effect of THAM + hydrocortisone + Metaraminol on endotoxin shock (*E. i*).

Discussion

Previous studies have shown that two characteristics of endotoxin shock in gs are oliguria and acidosis.[4,6-8] THAM has been shown to increase urine w and blood pH.[9-11] In this study both of these effects were realized. By mparing the control with the THAM-treated dogs, it may be seen that al-ough there was less hemoconcentration, a higher urine flow, and an alkalosis the dogs receiving THAM, their average survival time was less than that of e controls (10.6 hours versus 11.5 hours for the controls). FIGURE 3 shows e difference in blood pressure of the two groups. The control group showed initial rise in blood pressure and then a decline following the early endo-xin-induced hypotension, while the THAM-treated group showed a pro-nged hypotensive episode during THAM infusion, followed by a slow rise in blood pressure on discontinuing treatment. This same pattern was evide in the two groups graphed in FIGURE 8. Here the dogs were supported wi Metaraminol and hydrocortisone. The group receiving THAM showed



SUMMARY

Dog No.	Wt. (kg.)	Therapy Hydro.	+ Metarm.	Survival time (hr.)
1	13.0	300 mg.	5 mg.	17 days +
2	11.0		2.5 mg.	2.5 days +
3	10.5		12 mg.	7 hr.
4	9.5		2.5	6.5 hr.
5	11.7		2.5	5.5 hr.
6	9.5		16.5	8 days +
7	13.0		2.5	4 days +
8	7.8		2.5	7 days +
9	8.2		5.5	14 days +
10	10.0		4.2	3 days +

FIGURE 7. Effect of hydrocortisone + Metaraminol on endotoxin shock (*E. coli*).

fall in blood pressure after 5 hours, in spite of "pressor" therapy and, ev tually, included one less survivor than the group receiving no THAM.

Probably the most important fact is that while THAM caused the expec changes in urine flow and blood *p*H, it *did not* increase survival and, conside its hypotensive effect, THAM may actually be detrimental in endotoxin sh

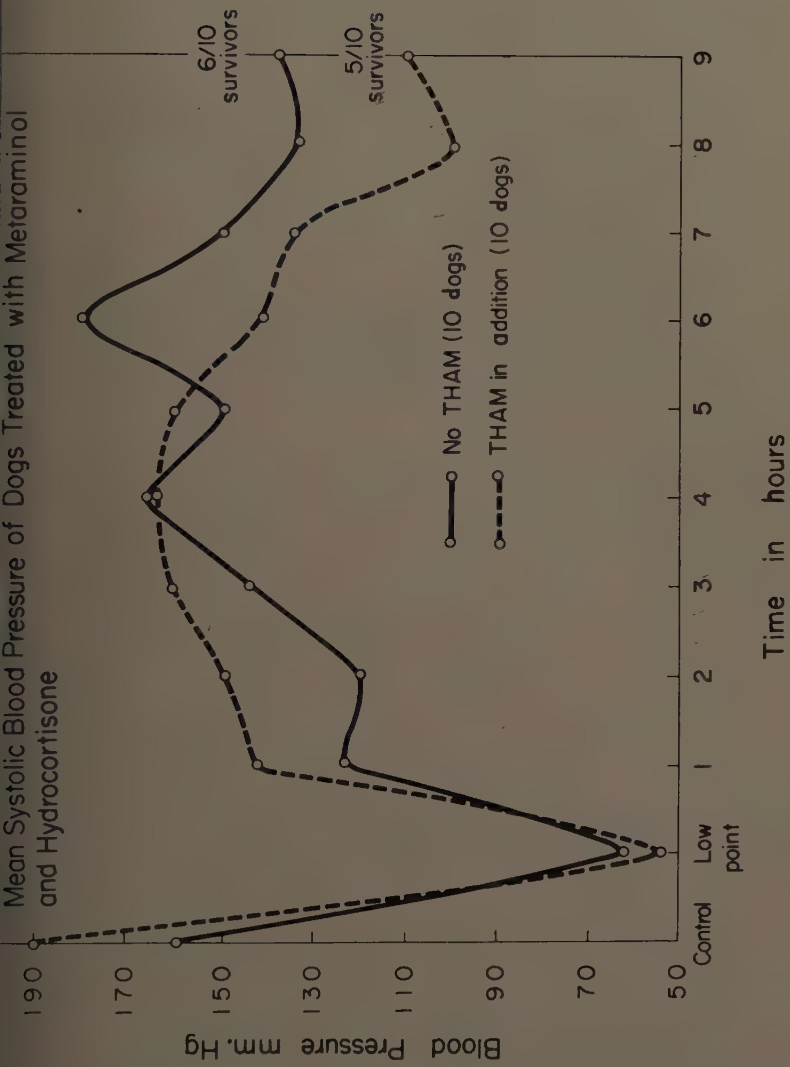


FIGURE 8. Mean systolic blood pressure of dogs treated with Metaraminol and hydrocortisone.

Summary

Forty-eight adult male mongrel dogs were studied in an effort to define effects of THAM in endotoxin shock. Although THAM was seen to allevi the acidosis, oliguria, and hemoconcentration that is commonly associated w this type of shock, its effects on blood pressure and peripheral vascular res ance were such that its use in hypotensive states may be contraindicated.

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THE EFFECT OF 2-AMINO-2-HYDROXYMETHYL-1,3-PROPANEDIOL ON THE ANURIA OF HYPOVOLEMIC STATES*

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he anuria accompanying hypovolemic states in general and hemorrhagic k in particular has been investigated by numerous authors. It is generally l that anuria is the result of a disproportionate diminution of the renal blood and a cessation of glomerular filtration with mean blood pressures below nm. Hg.[1,2,5] Also it has been observed that the lowest renal blood flows associated with the lowest *p*H, metabolic acidosis being the constant by- luct of poor perfusion.[2]

ith the availability for physiological use of an amine buffer 2-amino- ydroxymethyl-1,3-propanediol or tris(hydroxymethyl)aminomethane IAM),[3,4] which is a reversible hydrogen-ion acceptor forming compounds carbonic and other acids, the problem of anuria in hypovolemia seems well th investigating anew.

Methods

Iongrel dogs weighing 15 to 30 kg. were used. The dogs were anesthetized pentobarbital (35 mg. per kg.) and intubated. The saphenous artery and of one leg of each animal were used for recording arterial and central venous sure; the femoral artery of the other side was used for bleeding. All dogs ived heparin I.V. 3 mg. per kg.

small suprapubic incision was made and the ureters were cannulated just ve the bladder. The urinary flow was recorded by a drop recorder and a rated reservoir. All solutions were administered at a constant rate via the rnal jugular vein and recorded by a second drop recorder.

or the bleeding, the femoral artery was connected to a reservoir, the ht of which corresponded to the desired blood pressure. Adjustments in d pressure were made by adjusting the height of the reservoir. Mean blood sure was maintained at 40 mm. Hg during the period of bleeding. Arterial was determined at intervals at room temperature with a Beckman G *pH* er. Urinary *p*H was determined by the above technique and by the use H tape indicators.

Results

the control experiments urinary flow diminished with the onset of bleeding complete anuria developed, usually within 2 min. The arterial *p*H fell ually in all cases and, in many animals, reached levels below 7.20 during first hour of bleeding. In none of the dogs did the urinary flow resume re any of the shed blood was retransfused.

ne intravenous administration of normal saline, of a 0.6 *M* solution of sodium

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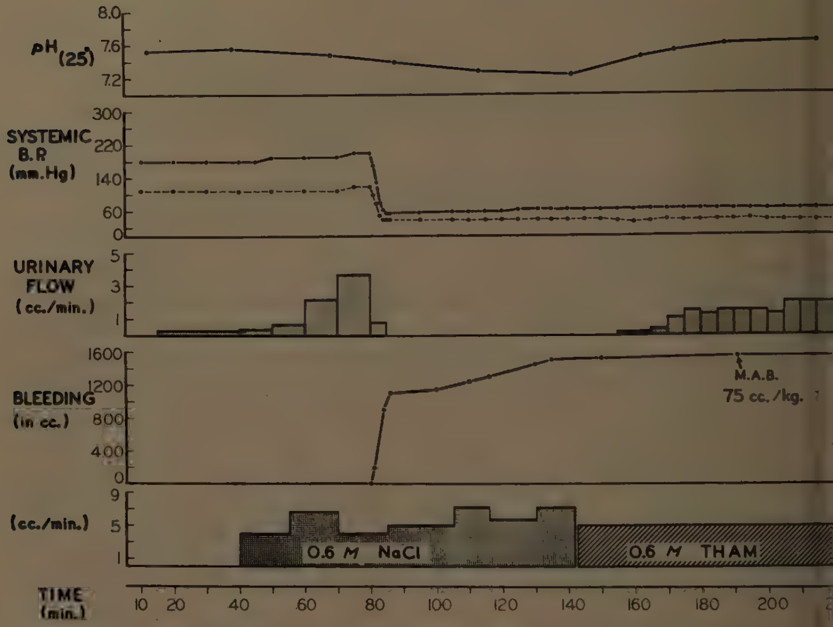

FIGURE 1. Anuria following bleeding despite continued administration of 0.6 *M* N also diuresis produced by the administration of THAM in the absence of any effect or blood pressure. The maximum amount of bleeding (M.A.B.) was 75.0 cc./kg.

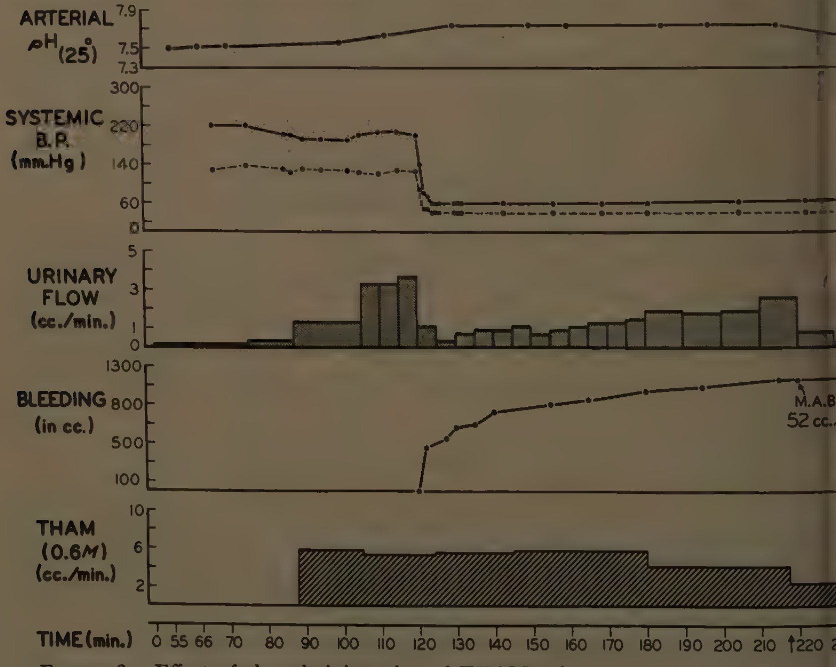

FIGURE 2. Effect of the administration of THAM prior to and during bleeding. the diuresis before bleeding, also the absence of anuria and the diuresis during hypovo hypotension. The maximum amount of bleeding (M.A.B.) was 52.0 cc./kg.

ride or of a 4.5 per cent solution of urea prior to bleeding, produced marked esis. However on bleeding, urinary output stopped abruptly despite the inued administration of these fluids at the same rate (FIGURE 1).

hen a 0.6 *M* solution of the buffer was administered prior to bleeding a use diuresis was obtained. When administration of the buffer was con- ed during bleeding there occurred a temporary diminution in urinary output increased with the continued administration of THAM, despite the fact the blood pressure remained at the hypotensive level. In many animals al diuresis was observed with the administration of THAM during the ovolemic hypotension (FIGURE 2). If anuria had been allowed to de- p with bleeding, either with or without the administration of fluids, the equent administration of THAM restored urinary flow in the absence of effect on blood pressure (FIGURE 1). Once urinary flow had been estab- d by the administration of the buffer, any decrease in its rate of administra- was immediately reflected in a decrease in urinary flow and, when adminis- on of the buffer was discontinued, anuria again developed.

though it was our aim to administer THAM at a rate that would keep the rial *p*H at a level of 7.6 during bleeding, this objective was difficult to eve, and the arterial *p*H varied at levels between 7.60 and 7.78. The exact unt of buffer that should be administered to produce urinary flow and the ionship between arterial *p*H and urinary flow during hypovolemic states ill to be determined. Upon discontinuation of the buffer, urinary flow eased at a rate that was out of proportion to the fall in *p*H and, in some , the arterial *p*H remained well above 7.50 when anuria developed (the still being in the hypovolemic state).

Conclusion

ese experiments demonstrate that THAM is effective in preventing or sing the anuria accompanying hypotension associated with large hemor- e. The mechanism by which this is brought about is presently being tigated.

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EFFECTS OF 2-AMINO-2-HYDROXYMETHYL-1,3-PROPANEDI DURING SHOCK AND CATECHOLAMINE ADMINISTRATION

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School of Medicine, New Orleans, La.

Much has been written about the reflex control of the cardiovascular sponse to exercise.[1-5] These studies have shown the marked degree of v constriction, the increment in heart rate, and the increase in myocardial tractility that occur to meet the needs of an increase in tissue perfusion. S changes were described by Cannon as a part of the fight or flight reflex. thermore, it is generally agreed that when the vascular column exceeds ci lating blood volume there is a closely similar response with an increase sympathetic tone and a decrease in parasympathetic tone.[1,6,7,8] This cha in tone is apparently elicited by increments in the concentrations of metabo in the area of the vasomotor center and by reflex impulses arising from "sinoaortic arch" receptors.

The metabolic alterations occurring with sympathetic stimulation ar increase in carbohydrate metabolism (hyperglycemia) with an increase duction of lactate, pyruvate, and other ions of organic acids.[9-12] Initi there may be a decrease in the arterial-venous (A-V) oxygen difference; h ever, an increase in A-V difference is usually seen in the later stages of stimulation. There is an increase in protein catabolism by the cells ar marked shift or loss of vascular water. The increase in protein catabo adds to the number of fixed acids found in the blood. The arterial *p*H been found to be near 7.0 in the terminal states of shock.[13,14] The los vascular water may be accompanied by an electrolyte shift in an attemp correct the marked tissue acidosis. However, Root and his associates shown that there is a striking correlation between *p*H decrease and the ir ments in plasma phosphate.[14] Since water is necessary for the hydrolys high-energy phosphates this loss of water may also occur with the us stored energy. The increment in plasma inorganic phosphate reporte Root *et al.* occurred when the blood volume decreased in excess of 20 per and increased progressively to higher values with further decrements in c lating blood volume. Watts and his associates reported increments in nephrine plasma levels occurring with a decrease in blood volume in exce 20 per cent and with further decreases in blood volume there was a fu increment in the plasma amine level.[15,16] Manger *et al.* reported si increments in epinephrine and also reported elevations in levarterenol hemorrhage.[17] Walton and his associates reported increments in pl catecholamine levels accompanying hypotension associated with hemorr and also reported a progressive acidosis associated with the incremen catecholamines.[18] Control of the sympathetic stimulation by pregangl sympathetic blockade and by the administration of hexamethonium red

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* The work described in this article was supported in part by grants from the Na Heart Institute, Public Health Service, Bethesda, Md., South Carolina Heart Associ Columbia, S. C. and the American Heart Association, New York, N.Y.

levels of the sympathetic amines and also limited the acidosis. The
onse to test injections of levarterenol was well maintained in the animals
minimal acidosis; however, in the animals with developing marked acidosis
e was a gradual decrease in the amplitude of the cardiovascular response
he test injections of levarterenol. Visscher and his associates were early
bserving a decrease in the vascular response to epinephrine under conditions
cidosis.[19] Recent clinical studies have reported the loss of vascular re-
siveness during acidosis.[20]

Methods

he studies were conducted in apparently healthy mongrel dogs.
each of the experiments anesthesia was provided by 10 mg./kg. of mor-
e sulfate injected subcutaneously, followed in 30 min. by 15 mg./kg.
sodium pentobarbital administered intravenously. Ventilations were
rolled by means of a Harvard respirator that provided positive pressure.
rial blood pressure was recorded from indwelling polyethylene cannula in
descending aorta by means of a Statham transducer (P 23-D). Venous
eters were passed by way of the femoral veins to a point near the right
m for intravenous injections. Prior to thorocotomy 5 mg./kg. of sodium
obarbital was administered. Isometric tension was measured by means
Walton-Brodie strain gauge arch.[21-23] The sutures for attachment of
strain gauge arch were placed approximately 15 mm. apart. The 2 feet
e arch were placed approximately 20 mm. apart. When the strain guage
was attached to the ventricle, the muscle between the 2 points of attach-
t was stretched by 33 per cent of the end-diastolic length. The ventricular
ractile force (VCF) changes that were measured and recorded reflect
nsic changes in the length tension relationship elicited by humoral and
ogenic changes.
he ascending aorta was isolated and the probe of a square-wave electro-
netic flowmeter was placed around the vessel.[24] The probe was fitted so
there was a slight constriction of the vessel. This procedure did not
ge the values of aortic systolic or diastolic pressure and has been found
to change the rate of flow through isolated vessel strips. Standard limb
s of the electrocardiogram were connected by means of needle electrodes.
rterial *p*H, whole blood CO_2 combining powers, CO_2 content, and lactate
d levels were determined from samples of arterial blood obtained anaero-
lly from the descending aorta.[25] A Beckman Model G *p*H meter equipped
a Beckman microelectrode part number 40311 or a Cambridge Research
el *p*H meter were used to determine *p*H. Frequent duplicate *p*H determi-
ns were obtained from the different *p*H meters as a check. Blood gas
rminations were obtained according to the methods of Van Slyke *et al.*
ate blood levels were determined according to the method of Baker and
merson.[26]
zygos flow" was established by occlusion of the superior and inferior
e cavae with umbilical tapes placed around each vena cava. The ends of
apes were then inserted through a rubber tube that was depressed lightly
st the vena cava and held securely in place with hemostats. Care was

taken to occlude these vessels completely with a minimal amount of press
to prevent venous spasm. In the animals receiving drug infusion or injecti
simultaneously, catheters were passed via the right and left femoral ve
Intra-aortic injections were made through a catheter passed from the fem
artery into the aorta to a level just above the diaphgram.

Results and Conclusions

The alterations in cardiovascular dynamics, autonomic tone, and metabol
associated with shock were outlined in the introduction. These experime
were designed to study the relationships and relative importance of such cha
on myocardial contractility.

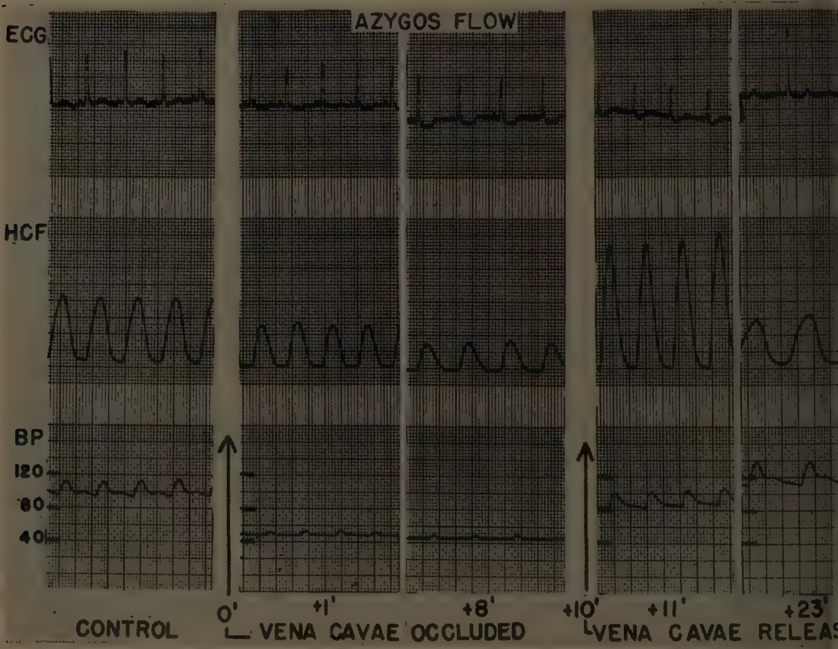


FIGURE 1. Effects of azygos flow on the electrocardiogram (ECG) Lead II, isom
tension of the cardiac muscle (HCF), and descending aortic blood pressure (BP) of the
The time interval between the heavy vertical lines is 0.2 sec. See discussion in text.

Effects of hypotension and hypoxia. In these studies acute hypotension
hypoxia were produced by limiting venous return to that supplied by
azygos vein (azygos flow). FIGURE 1 illustrates the changes in the ele
cardiogram (ECG) lead II, ventricular isometric tension (HCF), and art
blood pressure (BP) elicited during and immediately following this reduc
in cardiac output to nearly 20 per cent of normal for a period of 10 min. Du
this period of azygos flow a pronounced decrease in myocardial contract
accompanied the myocardial ischemia despite the marked increments in pla
catecholamine levels (from 3.0 to 91.8 μg./l. of plasma) reported by Be

his co-workers.[27] In each case, as illustrated here, at 1 min. after estab- nent of "azygos flow" there are changes suggestive of myocardial ischemia e ECG and depression of both diastolic and systolic tension. Eight minutes establishment of azygos flow there is definite S-T segment depression in ECG and further depression of isometric tension. Within 1 min. follow- release of the superior and inferior venae cavae there is an increment in ricular contractile force to values that double the preocclusion control. "rebound" increment in ventricular contractile force ranges from 50 to per cent above the preocclusion levels. After approximately 15 min. there secondary depression of isometric systolic tension. Catecholamine levels reported to average near 50 μg./l. of plasma during the initial rebound od and were reduced to values nearly twice normal (7 μg./l.) within 15 after the release of the venae cavae. Under these conditions the duration he "rebound" increment in ventricular contractile force was near 4.5 min. s myocardial contractility returned to values near or below preocclusion rol while the circulating catecholamine levels were still markedly elevated.

e blood pressure is a measurement influenced by stroke volume, heart , and peripheral resistance and, since isometric myocardial tension is more ted to energy metabolism, emphasis has been placed on this fundamental surement.

ith the reduction in tissue perfusion and sympathoadrenal stimulation e was a marked increment in blood lactate levels and a decrease in total , whole blood CO_2 combining power, and pH. The myocardial response test dose of 1 μg./kg. of levarterenol was completely blocked at the end he azygos flow period, and reduced to approximately 25 per cent of the cclusion response 5 min. after the release of the venae cavae. An in- sing responsiveness to the test dose of levarterenol was observed 15 and nin. after release of the venae cavae with the reduction of blood lactate ls and the return of total CO_2, CO_2 combining powers, and of pH to preocclusion levels.

other studies it was found that the rebound seemed to be more related to oval of the myocardial metabolites than to reoxygenation or to an increment he concentration of the catecholamines perfusing the coronary arteries.[28] se conclusions were based on left atrial perfusion during azygos flow with ed saline, 0.3 M solution of THAM in boiled saline, or with venous blood vn prior to the azygos flow experiment. Minimal increments in isometric olic tension were produced when left atrial pressure was raised to 6 cm. of by the atrial infusion of boiled saline. Similar infusions with the THAM tion increased ventricular contractile force to values near those produced release of the superior and inferior venae cavae. The atrial infusion of ous blood elicited comparable increments in myocardial contractility.

orrection of the acid-base derangements by the administration of THAM M infused intravenously during the period of azygos flow prevented the ked depression of the myocardium. The "rebound" stimulation of ven- ılar contractile force and arterial blood pressure lasted from 2 to 4 times uncorrected period of stimulation (8 to 16 min.). This longer period of ulation more closely paralleled the period of catecholamine elevation. lactate levels (9 to 14 millimole/l.) following the reduction in cardiac output in these animals more than doubled the concentration of lactate see the uncorrected animals. These results would seem to indicate that cor of the acid-base balance under conditions of acute reductions in cardiac put will result in better myocardial function and increase the limits of oxygen debt incurred. However, marked alkalosis or infusions of TH prior to the beginning of azygos flow seemed to prevent or markedly re the degree of sympathoadrenal stimulation. Longer periods of azygos

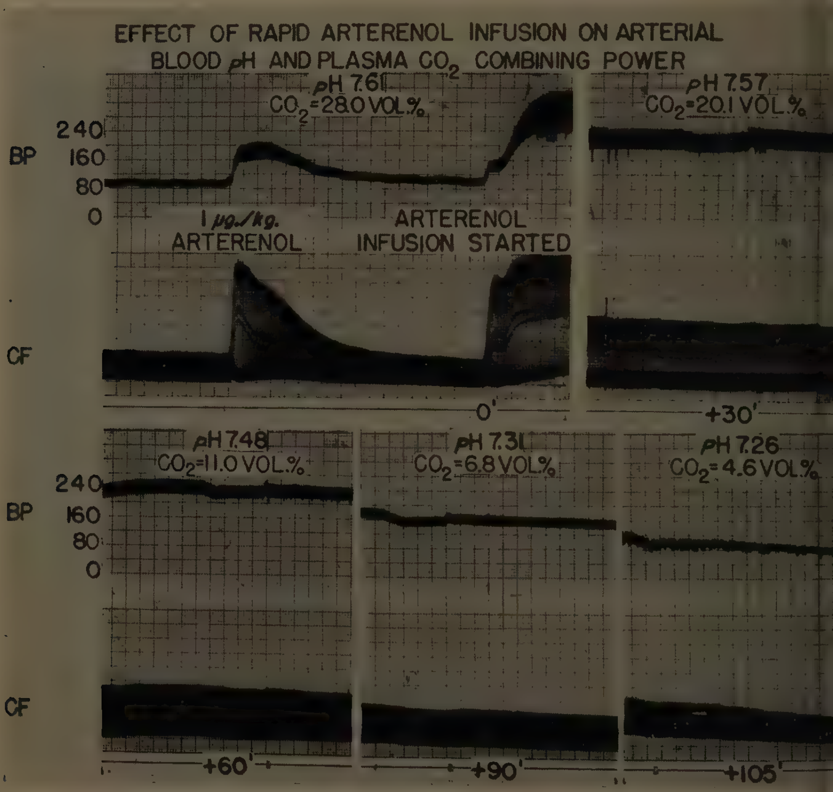


FIGURE 2. Effects of rapid levarterenol infusion on arterial blood pressure (BP) isometric tension of the cardiac muscle (CF). The time interval between the heavy ve lines is 20 sec. These changes are discussed in the text.

(30 to 45 min.) and overcorrection of the acidosis (pH 7.6 to 7.8) resulte apparent increases in central nervous system (CNS) anoxic damage. S changes in CO_2 concentrations are known to influence CNS vascular tone changes in blood chemistries elicited by THAM could be expected to incr CNS vascular resistance in the face of a decrease in cardiac output and contribute to the hypoxia. Experiments with extracorporeal circulation additional evidence to support this conclusion.

Rapid intravenous infusion of levarterenol and epinephrine: FIGURE 2 illustr the changes in cardiovascular dynamics, pH, and CO_2 combining power eli arked increments in blood levels of levarterenol. Comparable changes produced by epinephrine when this agent was administered by rapid venous infusion.[29] In the open-chest animal, hyperventilation was in- l by control of ventilation volume and rate. The *p*H changes primarily t the development of metabolic acidosis. The initial response demon- s the myocardial stimulation and increment in blood pressure produced μg./kg. of levarterenol under conditions of respiratory alkalosis. An venous infusion of levarterenol was started, and after 30 min. there was crease in ventricular contractile force and blood pressure despite the ase in concentration of the amine being infused. Notice that the decrease sponsiveness paralleled the increase in metabolic acidosis. Lactate blood were markedly elevated at the time of death. Vascular collapse and occurred shortly after the last section shown at 105 min. The animal ompletely refractory to large doses of the amines at this time. In closed- animals without artificial ventilation similar increments in lactate pro- on, reduction in total CO_2 content, and CO_2 combining power were ob- d. However, the marked degree of hyperventilation seen in these animals thought to be largely responsible for *p*H compensation. The relative for vascular collapse and death and the reduction in myocardial respon- ess were similar in both groups of animals, and they seem to depend largely te, concentration, and total amount of the amine infused.

e acidosis in these animals was accompanied by a marked loss of plasma ne and hemoconcentration. The hematocrit was frequently twice the ol value at the time of complete vascular collapse. Correction of the sis with THAM markedly increased the vascular and cardiac response to terenol and returned plasma volume to within normal limits. Control e acid-base balance disturbances remarkably increased the length of time e complete vascular collapse. THAM was found to be superior to other ring agents such as $NaHCO_3$ in the restoration of myocardial contractility. response to 3 per cent NaCl was somewhat less than the response to Na· $_3$, and sodium lactate was the least effective in the correction of acid-base gements or in the restoration of myocardial contractility. The adminis- on of the syntheticamines such as Aramine, Wyamine, and Ephedrine red the contractile force; however, there was a marked reduction in the nse to these agents.

usion of lactic acid in a manner similar to that used for the levarterenol ion elicited similar changes in acid-base balance, myocardial contractile blood pressure, and the response to injections of the sympathomimetic es. Correction of the additional acidosis with THAM produced a prompt n myocardial contractility and systolic blood pressure. With continued ion of lactic acid and THAM, acid-base values returned to near control. is time there was a secondary depression of myocardial contractility te the return of myocardial responsiveness to levarterenol test injections. reduction in contractility was thought to be due to the reduction in the evel of sympathoadrenal stimulation elicited during the period of acidosis.[30]

the case with levarterenol, infusion of THAM was more effective in re- ng myocardial function than the other buffering agents. While the acid- blood values were similar for THAM and $NaHCO_3$, 3 per cent NaCl and

sodium lactate were less effective in restoring myocardial function or no
acid-base blood values.[29]

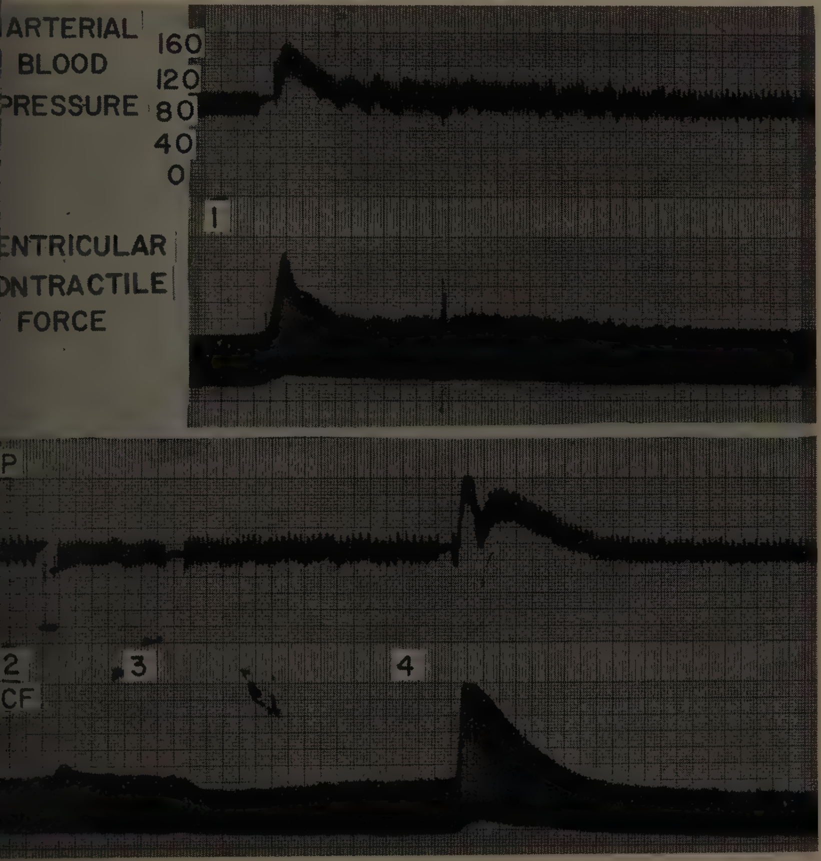
Hemoconcentration was a significant finding during the period of aci
regardless of the etiology.[31] This loss of plasma volume seemed to be la
responsible for the decrease in the vascular responsiveness. Frequently
blood pressure was well maintained the pulse pressure was markedly redu
Correction of acidosis with THAM resulted in a marked increase in
pressure and a reduction in hematocrit. This return of vascular vol
contributed substantially to the restoration of blood pressure and the incr
in the responsiveness of the vascular system to injections of levarterenol.

Effects of pH changes on the adrenal response and the increment in myoca
contractility elicited by adrenal stimulation. In several previous studies W
and his associates have shown that many substances are capable of stimula
the chromaffin cells and eliciting cardiovascular manifestations of cate
amine stimulation.[32,33] These investigators have used measurements of
changes of isometric systolic tension as an autoassay method of the degr
adrenal medullary stimulation. Injections of agents into the lower thor
aorta allows relatively high concentrations of the drug to reach the adr
glands while minimizing the amounts present in the general circulation.
thermore, the increments in isometric systolic tension were found to be in
agreement with the measured changes in plasma levels of the amines by
fluorometric method of Weil-Malherbe and Bone.

FIGURE 3 illustrates the procedure used to study the effects of acidosi
the adrenal medulla and on the myocardial response to adrenal stimula
In these experiments a catheter was passed from the femoral artery to a
just above the adrenal artery. This catheter was also used to measure art
blood pressure. Similar experiments were conducted in 6-open-chest ani
with midcervical vagosympathectomy and controlled respiration. At
point marked **1** in FIGURE 3 40 mg./kg. of lactic acid was rapidly injected
the arterial catheter (notice the absence of the pulsations in the blood pres
recording for the total time of the injection). An increase in myocardial
tractility was produced similar to that elicited by 1 μg./kg. of levarter
The irregularity of the blood pressure recording following the injection
due to the stimulation of respiratory movements. At **2**, 50 mg./kg. of TH
was administered in a similar manner. This agent also elicited a mild de
of cardiac stimulation and stopped the respiratory movements. At **3**
dose of lactic acid and THAM (40 mg./kg. LA and 50 mg./kg. THAM)
combined and given in 1 injection. Note that in this case there is a depres
effect on the heart and a lack of adrenal stimulation, since the THAM w
have prevented the marked *p*H change. At **4** the intra-aortic injectio
lactic acid was repeated; however, in this case 50 mg./kg. of THAM
administered intravenously at a site in the inferior venae cavae near the
atrium. The increment in ventricular force elicited by the adrenal stimula
was substantially greater when the *p*H reduction of the blood perfusing
heart was prevented or reversed.

FIGURE 4 illustrates a continuation of the previous study. In these ani
studies were made of the effects of acute changes in *p*H on the myoca

nse to catecholamine stimulation. Cardiac output (CO) was monitored eans of a square-wave electromagnetic flowmeter. The first response, **1**, btained following an intravenous injection of 0.5 μg./kg. levarterenol and



URE 3. Effects of acidosis on the adrenal medulla and on the myocardial response to al stimulation. At **1**, 40 mg./kg. of body weight of lactic acid was rapidly injected into rta just above the adrenal artery. At **2**, 50 mg./kg. of THAM was injected into the catheter. At **3**, the dose of lactic acid and THAM were combined and injected simul-usly. At **4**, the lactic acid was given in the aorta, so that adrenal stimulation was elic-nd the THAM was administered into the inferior venae cavae near the right atrium. me interval between the heavy vertical lines is 20 sec. The experiments from which this ation was obtained are discussed in the text.

g./kg. of lactic acid given simultaneously in the same syringe. The d response, **2**, illustrates the changes obtained following an injection of 0.5 g. of levarterenol, 40 mg./kg. of lactic acid, and 50 mg./kg. of THAM taneously. In every case there was an obviously greater increase in etric systolic tension when THAM was added to the levarterenol and acid mixture. Concomitantly there was an increase in isometric diastolic

tension. When THAM was added there was an initial decrease in blood
sure, and a marked increase in cardiac output. At **3** a volume of no
saline equal to the volume of fluid given at **2** was injected intravenousl

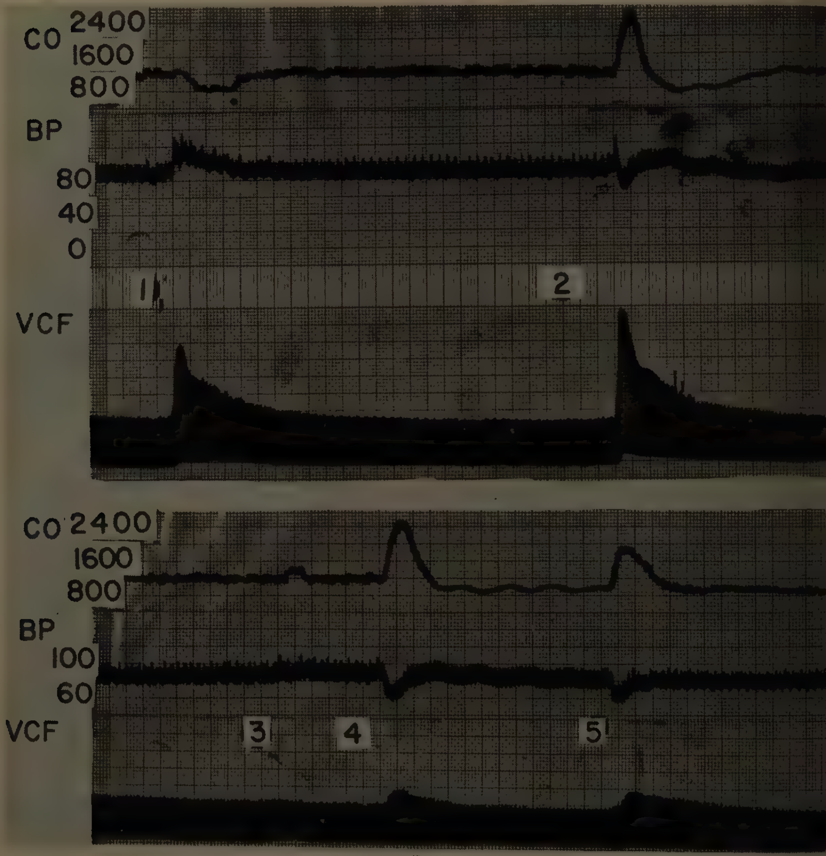


FIGURE 4. Effects of acidosis on the myocardial response to levarterenol. Cardiac
put (CO) was measured from the ascending aorta by means of an electromagnetic flowm
Blood pressure (BP) was obtained from the descending aorta. Isometric tension (VCF
measured by means of a strain gage arch sutured to the right ventricle. At **1**, 0.5 μg
of levarterenol and 40 mg./kg. of lactic acid was administered by means of a single inje
into the inferior vena cava near the right atrium. At **2**, the same dose of lactic acid
levarterenol was combined with 50 mg./kg. of THAM and given as a single caval injec
At **3**, a volume of normal saline equal to that given at **2**, was administered in the same ma
as the previous injections. At **4**, 50 mg./kg. of THAM was similarly administered.
nitroglycerin (0.05 mg./kg.) was given intravenously. The time interval between the h
vertical lines is 20 sec. See text discussion.

the same manner as the drug injection. This procedure failed to elicit
marked increase in cardiac output seen with the drug combination.
THAM, 50 mg./kg., elicited a change in blood pressure and cardiac ou
closely similar to that seen with the drug combination at **2**. At **5** the co
bution of the hypotension was investigated by means of an injection of n

rin 0.05 mg./kg. intravenously. The volume of fluid administered with itroglycerin was only one sixth of that given at **2** (5 cc. as compared to), but the increment in cardiac output was substantial. The combina- f hypotension and fluid volume injected can largely explain the increment rdiac output seen with the injection of THAM.

Discussion

er periods of hypotension and reduced cardiac output despite normovole- ondition one might expect myocardial failure as a contributing factor to ant shock.[18] Wiggers has suggested that myocardial failure seemed to e major contributing factor in approximately 80 per cent of the cases of ersible hemorrhagic shock following return of adequate blood volume.[1] tudies outlined in this report and other studies emphasized the importance equate removal of metabolites and restoration of acid-base balance as ary factors in determining the isometric systolic tension developed by the ardium in response to endogenous sympatho-adrenal stimulation or to nously administered cardiotonic sympathomimetic amines such as levart- l.

ssically, the relationship that exists between end-diastolic length and on and the force of contraction of the myocardium have been recognized. hermore, the important increases in the length-tension relationships that with sympathoadrenal stimulation have been documented as a means eeting an increase in cardiac output and tissue perfusion during periods ercise.[22,34] However, in disease states with prolonged period of sympatho- al stimulation, acidosis and a decrease in myocardial contractility are to occur despite a marked increase in the circulating levels of the catechol- es.[7]

on and Piatnek have suggested that myocardial metabolism might be ed into three general phases: (1) energy liberation, (2) energy conservation orage, and (3) energy utilization.[35] The change in isometric tension oc- ng with systole is proportional to the maximum work capacity of the le. The amplitude of this change is mainly related to energy utilization arallels the change in free energy.[36] Root and his associates have shown there is a striking correlation between pH decreases and increments in a phosphate.[14] This increase in phosphate could be related to a reduction red energy due to a deficit in energy liberation. Furthermore, the hyper- mia, the increased production of pyruvate and lactate, with a greater ase in lactate, would seem to indicate that under the conditions of sym- adrenal stimulation the energy liberated is largely derived from anaerobic hydrate metabolism.

e oxygen debt that the heart can incur is believed to be limited since the muscle is embedded in well-developed oxidative machinery.[37] Carbon le, the end product of aerobic metabolism, is markedly depressant to the ardium. This gas is also liberated as a result of the bicarbonate buffering tic acid. Lundholm has presented excellent evidence that the vasodilator n of epinephrine is related to the release of CO_2 from the bicarbonate ing system.[38] Since the pK_a of lactic acid is near 3.5, this agent, as it is d, is immediately converted to lactate. This change seems to be largely at the expense of the bicarbonate buffering system. These changes in bicarbonate buffering system, CO_2, and hydrogen ions would lead to a re tion in energy liberation from both aerobic and anaerobic metabolism accor to the law of mass action.

Nahas and his co-workers have reported that correction of respira acidosis results in a decrease in sympathetic stimulation as evidenced b decrease in the plasma catecholamine levels despite the continued presenc hypercapnia.[28] In these studies the stimulating effects of lactic acid on adrenal medulla were shown. In addition, the depression of myocar contractility by lactic acid and the reduction in the myocardial respons administered levarterenol or endogenously released catecholamines were sh under conditions that would produce myocardial acidosis. The lack of adr stimulation by lactic acid when the hydrogen ion concentration was contro with THAM was also demonstrated. These results would emphasize importance of changes in *p*H on the sympathoadrenal system and the resp of the cardiovascular system to these agents. Apparently acidosis is a stim to the sympathoadrenal system. When acidosis is not severe the myocard and vascular system respond to the presence of increased catecholamine le However, if the acidosis is severe the depressant effects of this condition myocardial energy metabolism reduces the myocardial response to catec amines and prevents this organ from pumping an adequate supply of oxygena blood to the tissues.

These results would seem to indicate that since the body relies on sympa adrenal stimulation when there is an immediate need for an increase in car output, the greater increase in myocardial contractility under condition acid-base balance would be very important. The lack of adrenal stimula in the presence of increased lactate that has been buffered might indicate overcorrection of acidosis could deprive the animal of adrenal stimulation a time when this stimulation is needed. Since the supportive therapy many emergency states depends on the myocardial response to sympatho metic amines, the recognition and correction of acidosis is of crucial importa in gaining added time to correct the etiology of the shock condition.

Summary

Acidosis is a stimulus to the sympathoadrenal system and depresses myocardial response to catecholamines. 2-Amino-2 hydroxymethyl-1 propanediol is more effective than other buffers in restoring the myocar response to catecholamines. Overcorrection of the acidotic state seems depress sympathoadrenal tone, elicit cerebral vasoconstriction, and red total peripheral resistance. The reduction in the degree of hypertension the increase in myocardial contractility elicited by the combined adminis tion of levarterenol and THAM could markedly increase cardiac out Other important changes are discussed, such as the plasma volume char with acidosis and correction of this condition.

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E USE OF AMINE BUFFERS IN CARDIOVASCULAR SURGERY*

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esent interest in the physiological use of the trihydroxyamine buffer that
e chief subject of this monograph began with the publication of Nahas'
.[1] Previous to this, I, like most other biochemists, had used this buffer
nzyme determinations and in cell metabolism work. Factors that en-
aged my colleagues and me to study the use of Tris buffer further were:
hat, although no one knows why, it seems to be exceedingly stable, a sort
 "organic metal"; (2) the indication that it may be an intra- as well as
xtracellular buffer; (3) the fact that, in a sense, it helped the kidney per-
 part of the function of the lungs, namely, that of carbon dioxide elimina-
; (4) that it is a strong buffer in the physiological range; (5) that it is, to
best of our present knowledge, not deaminated or oxidized to aldehydes
cids in the body; (6) that a sterile preparation was available†; (7) that
 appeared to be a need for an improved means to treat the patient who is
otic largely because of myocardial failure; and (8) our first applications in
cal work were encouraging.

 seemed to us that if this organic amine did not complicate the already
plex elements involved in open-heart surgery, such as the regulation of
ing times and blood gas exchange, it might prove useful in dealing with
occasional temporary and reversible postoperative acidosis encountered in
 surgery. Our experimental work was largely oriented to determine if it
d be used in these types of patients. Some additional clinical work was
 to learn more about dosage and blood levels and to see if it could be other-
 useful. In particular we thought it might be valuable in countering the
ound and rapidly developing acidosis encountered in cardiac arrest; in this
ection, we were eager to be sure that it would not hamper defibrillation.

e have instrumentation for the intermittent automatic or continuous re-
ing of blood pH, pO_2, oxygen content, carbon dioxide content, and tem-
ture, both in the heart-lung machine and in the patient in the postopera-
period, and methods for measuring blood amine levels either continuously
termittently.

Results

otting time. In order to determine if Tris would speed the excretion of
rin or otherwise change the clotting time, dogs were given fixed doses of
rin and their clotting time was followed. After the curve had been es-
shed for a given animal, it was allowed to recover for several days and the
was repeated. This time, however, a Tris infusion was started shortly
 the heparin was given. The results (FIGURE 1) permitted us to conclude

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ational Heart Institute, Public Health Service, Bethesda, Md., and by research grants
the Alabama Heart Association, Birmingham, Ala.
bbott Laboratories, North Chicago, Ill.

that Tris would not speed the excretion of heparin, as we thought it mig and that it would not complicate control of our anticoagulant regime, wh is necessary in open-heart surgery. Furthermore, it was shown not to interf in protamine titrations.

Potentiation of pressor effects. After correcting acidotic patients with T there is a potentiation of the effects of pressor agents. Deamination of ph ethylamines by rabbit liver homogenate was found to be somewhat lower the presence of Tris buffer than in the presence of phosphate buffer. For t reason we compared the pressor responses to tyramine in cats before and a the administration of Tris or Tris chloride to determine if amine oxidase

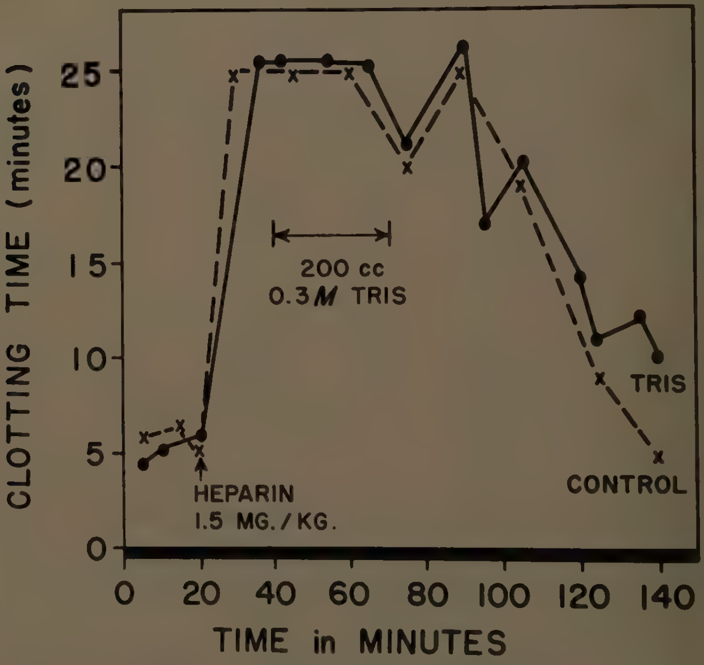
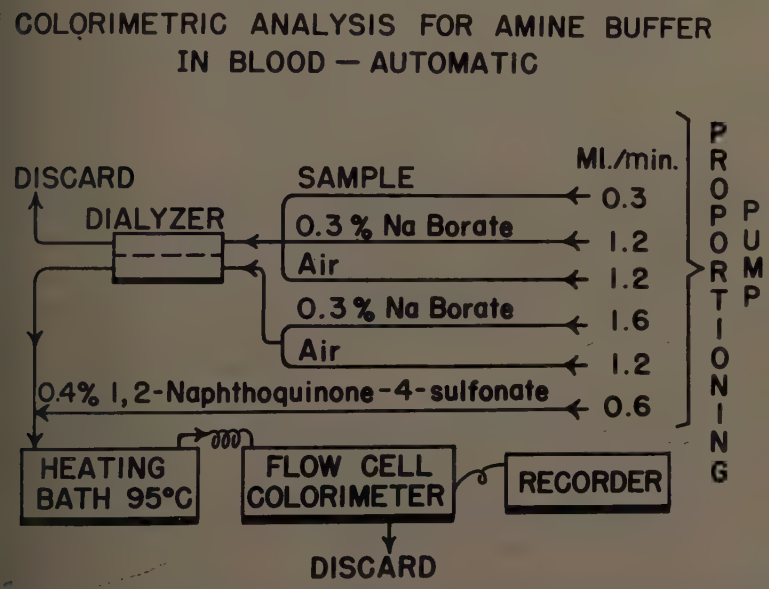


FIGURE 1. Clotting time and Tris infusion.

inhibited *in vivo*. We were unable, in a number of experiments, to find potentiation by Tris of the tyramine pressor response in cats, although it readily demonstrated after a small dose of Catron, a known MAO inhibi We therefore concluded that, although there might be slight suppression amine oxidase activity *in vitro*, there is no significant suppression *in vivo*.

Analysis for blood amine levels. H. Rosen of the Walter Reed Army In tute of Research, Washington, D. C., kindly provided us with his method measuring urinary amine levels, which we find useful, but we were unable locate a satisfactory method for determining Tris levels in blood. The ini blood analyses were conducted using a modification of the sodium phen method for ammonia, which was modified to decrease the sensitivity to monia and to increase the sensitivity to amine. This method was automa

Tris much as described by Logsdon for ammonia.[2] In searching for a r method for blood Tris analysis, we adapted the old amino-acid method described by Folin and improved by Frame *et al.*[3] We found that it could ed for measuring blood amine levels and, furthermore, that it was easily ted to an automated procedure. This method is shown schematically in RE 2. The color responses follow Beer's law, as shown in FIGURE 3, which eproduction of a recording from the analytical machine. Twenty samples e analyzed per hour. Recovery of Tris added to blood or plasma is com- below about 50 mEq. per liter; above that, it falls off to about 80 per at 100 mEq./l. We have never observed a blood amine level above 50



GURE 2. Arrangement of proportioning pump, dialyzer, heating bath, flow cell colorim- and recorder (Autoanalyzer) for continuous or intermittent analysis of blood for Tris. .4 per cent naphthoquinone reagent must be prepared fresh daily; the 0.3 per cent n borate is stable. The colorimeter is equipped with 625 mμ filters.

./l. Blood samples should be kept cold and the plasma frozen if analysis ot be performed at once. Although the naphthoquinone procedure proved actory for this work, there is need for more sensitive and specific pro- res than have been developed thus far.

Tris is added to whole blood, and the cells and plasma are analyzed sep- ly, it is found that a small part of the Tris ion rather rapidly disappears the plasma and is, perhaps, absorbed on the erythrocytes. If human l is incubated at 37° C. with Tris chloride (*p*H 7.1) the Tris level remains ant in both cells and plasma for several hours. We have not been able monstrate Tris in erythrocytes exposed to Tris either *in vitro* or *in vivo*, separated, hemolyzed, and analyzed. However we are puzzled by these vations.

To determine the maximum blood levels that could be reached by infu
Tris into animals, 0.3 M Tris (Abbott's THAM with NaCl and KCl) was
fused at a constant rate while blood levels were continuously recorded.
URE 4 shows such an experiment conducted with an animal having its r
arteries ligated. If all of the Tris (36.3 gm.) had stayed in the blood, the l
would have been about 215 mEq./l. of blood. If the Tris had been equ
distributed throughout the body, the level would have been 300 divided
14, or about 21 mEq./l. of dog. If the Tris had been distributed on the b
of body water, the blood level would have been somewhat higher than t
The highest blood level actually recorded was 34 mEq./l. Repetition of
experiment in an animal having kidneys with normal blood supply gave bl

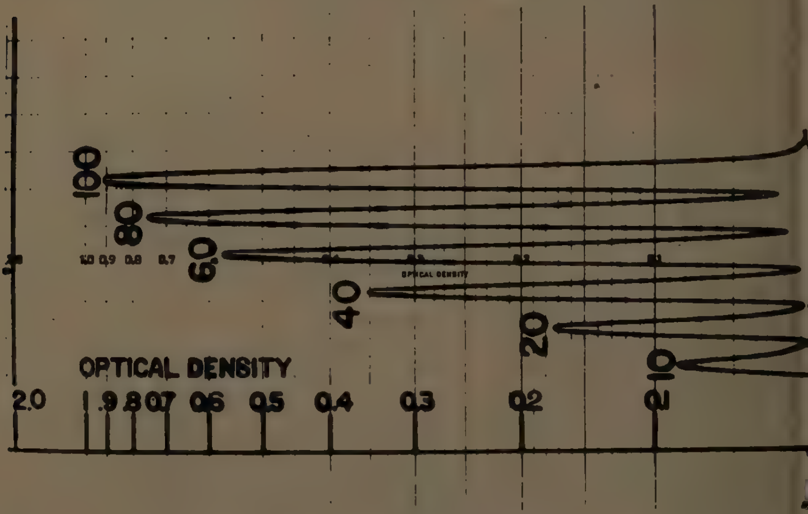


FIGURE 3. Concentration versus optical density. The standards used are Tris chl
(pH 7.4). Concentrations are in mEq./l. The apparatus can be set to give full-scale rea
with the 20 mEq./l. standard. Base-line readings on control (before Tris) blood san
must be obtained if low concentrations of Tris are present.

levels that were strikingly similar (FIGURE 5). These figures also demonst
that blood pH measurements give no information concerning blood Tris le

Similar blood measurements have been done following smaller doses of
After the administration of 5 cc./kg. of the 0.3 M Abbott Tris, blood level
approximately 2 mEq. were found. As we shall see later, significant buffe
occurs with doses as small as 5 cc./kg., or at blood levels of 2 mEq./l.

The analysis of several dozen blood samples from patients showed that,
erally speaking, blood levels of about 5 mEq./l. were attained, but tha
some patients there was a delay in observing a rise in blood level and in ot
there was an abrupt rise. The highest level observed was 14 mEq. in a 65
man who received 1300 cc. of 0.3 M Tris over a three-hour period. Bec
the blood level resulting from a given dose is not readily predicted, we
reduced initial doses to not over 5 cc./kg.

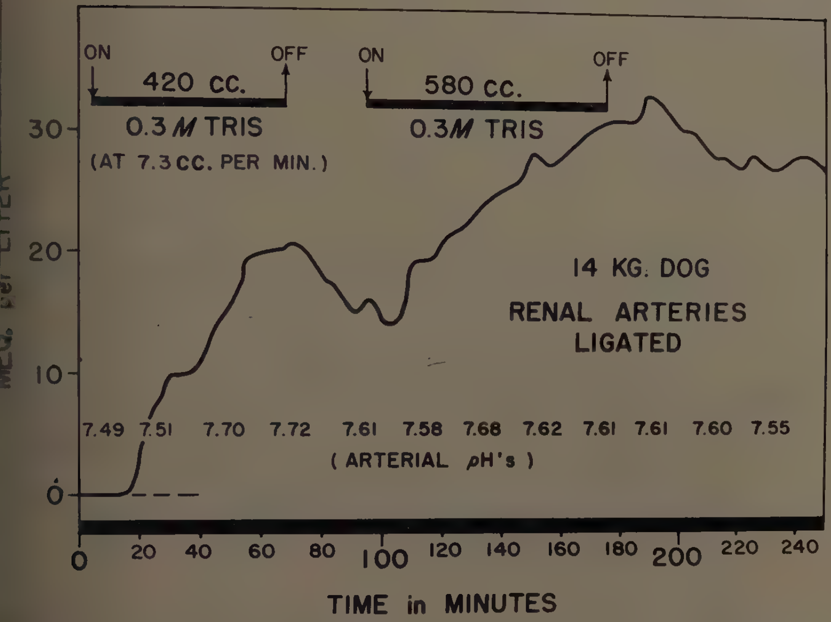
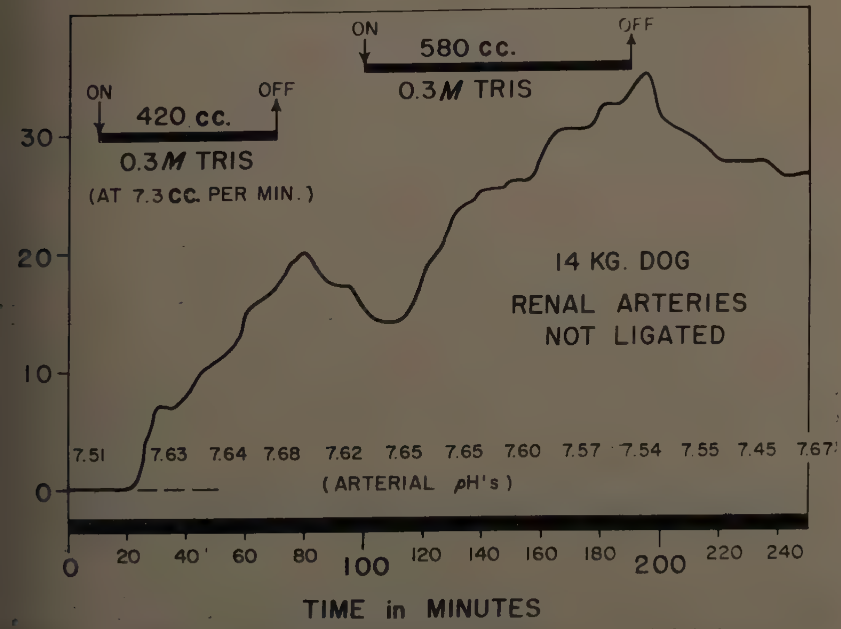


Figure 4. Continuous analysis of arterial blood Tris levels with Tris infusion at a con-
nt rate.



igure 5. Continuous analysis of arterial blood Tris levels with Tris infusion at a con-
t rate.

Speed of neutralization. The rate of disappearance of a rapidly injec bolus of Tris buffer was measured by means of an arterial platinized platin electrode.[4] The platinized electrode, which can be made small enough to pl directly into arteries, provides a convenient method of detecting rapid changes in the arterial stream, for it changes in potential with *p*H. FIGUR shows an oscillographically-recorded potential change following the rapid travenous injection of 1 cc./kg. of 0.3 *M* Tris buffer into a cat. It can be s that the buffer is not neutralized in passing through the lungs, where it mi pick up some CO_2, but is neutralized by the time it makes one complete culation. The maximum *p*H found at the peak of the curve was 8.39. cause of the late change of potential recorded by the electrode is not establis as yet, but it occurs uniformly after the rapid injection of Tris and appear coincide with an increase in blood Tris level and of *p*H. This change is m too late to be related to recirculation. Curves obtained after the rapid in tion of 0.3 *N* sodium carbonate solutions were similar to those obtained a the injection of 0.3 *M* Tris, including the late drop in potential of the rec ing electrode. We wonder if Tris and sodium could be adsorbed on the terial wall and then slowly released again.

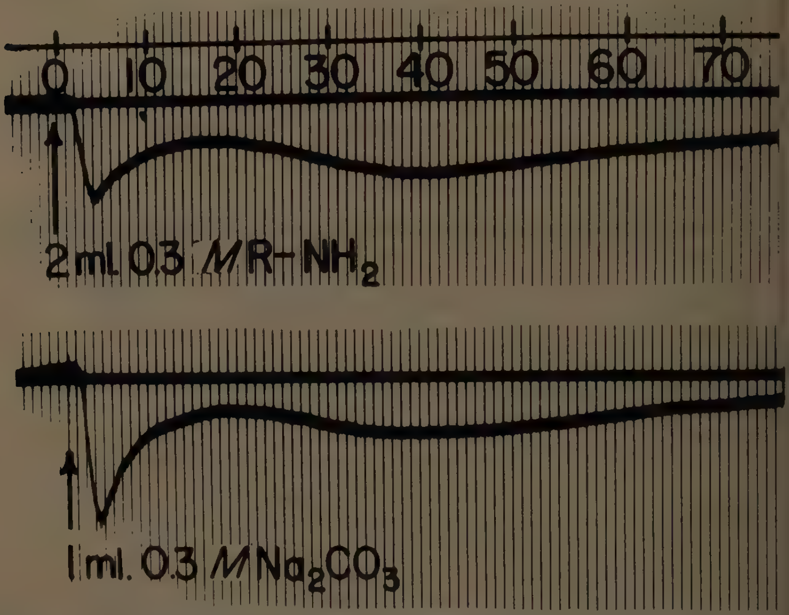
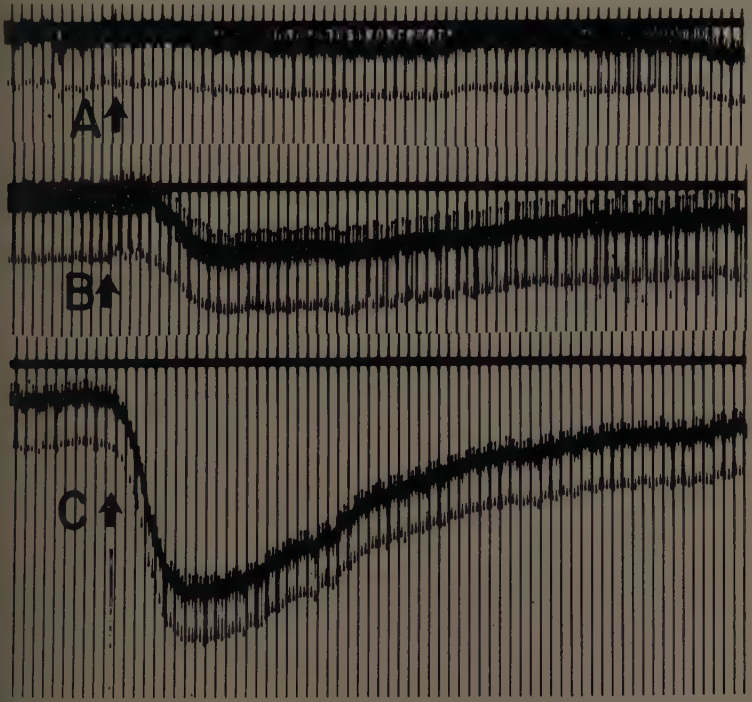


FIGURE 6. Potential change from an arterial platinum electrode. A platinized plati electrode was placed in the artery and its potential, with respect to a silver plate on the s was recorded. Full-scale sensitivity is approximately 50 mv. The vertical lines are 1 intervals; the upper scale is in seconds.

Arterial oxygenation. It was observed in animals that arterial oxygen s ration decreased after Tris buffer was given; this seemed to be related to depression of respiration that was routinely observed and that is the sub

papers and discussion in this monograph. Nevertheless it seemed conceiv-
le that small right-to-left pulmonary shunts might be opened by the amine
ffer. T. D. Darby, of Tulane University, New Orleans, La., in a recent
it to Birmingham, suggested this possibility.

We had previously observed that hydrogen is cleared from intravenously
ected solutions of hydrogen and that this was a sensitive test for right-to-
t shunts.[5] FIGURE 7*A* shows a test performed before the administration of
s to an animal and *B* shows a test performed afterwards. Tracing *C* is
wn to indicate the response of the electrode to a very small dose of gaseous
rogen given into the trachea. From curves of this kind we have concluded
t small right-to-left pulmonary shunts are produced in dogs. If these
nts persisted after the administration of oxygen it would follow that a high
rial pO_2 could not be obtained following the administration of oxygen.
eriments were done to answer this question.



IGURE 7. Potential change from an arterial hydrogen electrode. The upper tracing
shows the lack of potential change in an arterial electrode (silver reference) when hydro-
saturated saline was injected intravenously. Curve *B* was obtained after infusing Tris
er and repeating the test. Curve *C* shows the response obtained following a single injec-
of gaseous hydrogen into the trachea. The animal was breathing air throughout.

IGURE 8 shows pO_2 continuously recorded from the femoral artery (by
nping arterial blood through a constant-temperature stirred cuvette and
rning the blood to a femoral vein) before and after the administration of

Tris buffer. It can be seen that arterial pO_2 remains high after the admin
tration of Tris chloride and that when the animal is breathing oxygen (or
respirator in this case) these shunts must close. We conclude that the
pression in arterial oxygenation observed in the earlier experiments were
primarily to the depression of ventilation induced by Tris. Respiration
animals receiving large doses of Tris can be completely stopped with minu
doses (as little as 5 mg.) of Surital.

Brain oxygen availability. Since the major factor regulating cerebral blo
flow is the carbon dioxide pressure of the blood, it seemed desirable to det
mine the influence of Tris infusion on cerebral oxygen availability.[6] We w
dered if cerebral oxygenation could be impaired even in the presence of

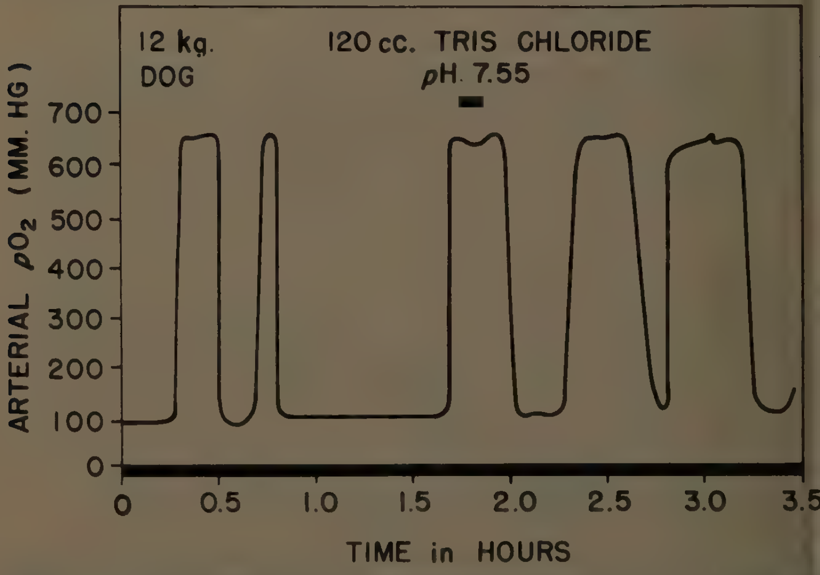


FIGURE 8. Arterial oxygen tension. The arterial pO_2 was recorded with a Clark oxy
electrode in a stirred anaerobic cuvette maintained at 37° C. During the time indicated
the bar at the top of the graph the buffer was infused intravenously.

arterial pO_2 of more than 600 mm. Chronically implanted platinum catho
polarized at −0.6 v, are very sensitive to changes in tissue oxygen pressure

Platinum electrodes, insulated up to the tip, were implanted in the brain
a cat and allowed to heal for 5 weeks. A silver rod fixed to the skull ser
as a reference electrode. After allowing 20 min. for the current to bec
stable, 1 cc./kg. of 0.3 *M* Tris was rapidly injected intravenously. The
gen availability dropped to about 60 per cent of its previous level and requ
about 15 min. for return to its previous reading. A brief inhalation of
rapidly returned the reading to normal. When the experiment was repea
while the animal was breathing 5 per cent CO_2 and 95 per cent oxygen (w
gave a current flow 1.9 times that obtained on air) an even greater change
observed following the injection of buffer. This is shown in FIGURE 9. S
the effect lasts considerably longer than it takes to neutralize a single injec

buffer, it may be that the buffer penetrates the vessel walls and drops the CO_2 locally more than it does in the blood. The oxygen supply to the brain creases during rapid infusion of Tris, even though the arterial pO_2 remains gh.

This effect on cerebral circulation could possibly lead to serious consequences Tris buffer is administered too rapidly under conditions in which cerebral ygenation or circulation is already impaired.

In studies of hemorrhagic shock, proceeding at the present time, we have served that administration of the amine buffer *intravenously* sometimes ecipitates collapse of the animal, while administration of a similar dose of ffer *intra-arterially* into the descending aorta improves blood pressure and diac output.

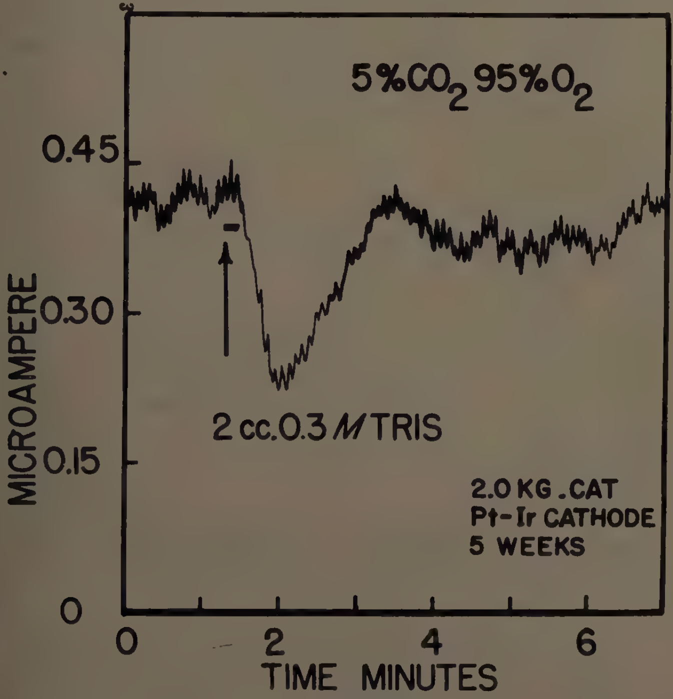


Figure 9. Brain oxygen availability.

Buffered surgical arrest. The use of potassium citrate as an agent for arrest- the heart for surgical purposes has been shown to result in focal necrotic mage to the myocardium.[7] The mechanism of this damage has never been nonstrated clearly.

n studying this phenomenon, isolated dog hearts perfused by the Langen- f technique were used. "Anoxic arrest" produced by merely clamping the ta was compared with potassium citrate arrest.[8] It was found that the of the coronary venous blood that appeared after release of the aorta was er in potassium citrate-arrested hearts than in anoxic-arrested hearts (FIG-

URE 10). Intermittent anoxic arrest has been shown to be safer than citra arrest. This suggested that control of the myocardial pH with buffers may desirable. FIGURE 10 also shows that Tris citrate, injected instead of pota sium citrate, results in a stabilized pH during the acirculatory period.* The buffered isolated hearts, left without circulation for 30 min., were easily turned to normal rhythm and to what appeared to be good contractility.

These experiments were extended to the arrest of hearts for 45-min. peric in dogs whose circulation was maintained by a heart-lung machine.[9] Varic salts of Tris were compared by injecting them into the aorta proximal to t clamp. Tris citrate, chloride, gluconate, and phosphate were tested genera

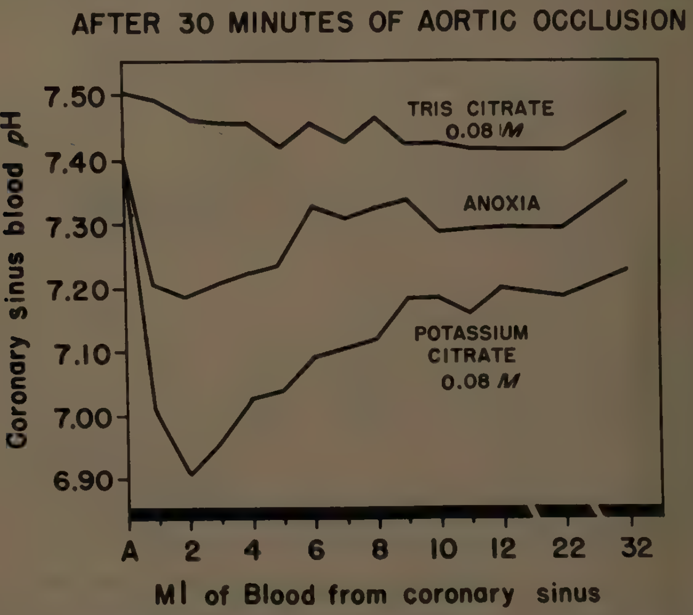


FIGURE 10. Changes in pH during elective cardiac arrest. After the period of arr the aorta was released, coronary sinus blood was collected in consecutive 1-ml. portions, the pH was determined.

in 0.03 M concentration by diluting 0.3 M solutions with arterial blood. T chloride and Tris phosphate, as might be expected, did not induce arrest, perhaps prolonged the period in which the acirculatory heart beat. Tris rate produced arrest, but more slowly and less completely than potassi citrate. Tris citrate with potassium (10 mEq./l.) induced prompt arr Altogether, 16 experiments were done, and although some surviving anin were obtained, it was apparent that in the postoperative period the card output was low, and the hearts, especially the right ventricle, were not fu tioning well. Mixed venous oxygen content, our main criterion of card output, recorded continuously, fell to 40 per cent or below. Sections of hearts showed various types of damage. Tris citrate- and gluconate-buffe

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* This research was done with the assistance of Joe Lyons and Paul Stabler.

arts fared best, at least one of each showing no pathology on section. With
ded potassium, diffuse and focal necrosis were observed.

Experiments of this kind are difficult to do and to interpret unless the differ-
ces are great, which they were not. One of the difficulties is distribution.
for example, dye is injected into the coronary circulation, as was done in
se experiments, it is easy to demonstrate that large areas are not stained.
nce one cannot be sure where the drug or buffer went in a given case.

Parenthetically, it may be added that anoxic arrest is a mystery. If sodium
anide is injected proximal to the aortic clamp, the heart remains pink, as
uld be expected, but it continues to beat for up to 10 min., generally with
ceful contractions. Sections of the left ventricle of these hearts were re-
rted as unremarkable or negative by the pathologists. The hearts injected
th Tris phosphate beat longer than with the other Tris salts or with anoxic
rest. At present it appears that buffered anoxic arrest offers no advantage
er anoxic arrest. We intend to continue these experiments since continu-
s perfusion of coronary arteries during aortic valve surgery cannot always
performed as desired, and means for protecting the acirculatory heart are
eded.

Defibrillation. The metabolic and respiratory acidosis that develops follow-
cardiac arrest may in itself do damage. This acidosis usually develops
ther during cardiac massage because it is seldom possible to obtain a satis-
tory cardiac output by this means.

To evaluate the use of Tris buffer in cardiac arrest, thoracotomized dogs were
jected to cardiac fibrillation by a 10-v shock. After 3 min., massage was
gun and attempts at electric defibrillation were made every 5 min. Ten
ntrol dogs received 5 cc./kg. of saline at the end of the 3 min., while 11 other
gs received a similar amount of 0.3 *M* Tris. The *p*H of blood samples taken
m the vena cava, the pulmonary artery, and the femoral artery were meas-
ed every 4 min. Altogether, 770 samples were measured (Radiometer *p*H
eter).

From FIGURE 11 it may be seen that the *p*H continues to drop throughout
period of massage. FIGURE 12 shows that animals surviving fibrillation,
ssage, and defibrillation tend to correct their acidemia as would be expected.
The more gradual drop in *p*H in the animals receiving buffer is shown in
URE 13. Note also the wide AV *p*H difference in these buffered nonsur-
ing animals, as was also observed in the nonsurviving control dogs. This
of course, indicative of a low cardiac output. After 20 min. of resuscitative
rts, the arterial *p*H in these buffered animals is still about 7.35. FIGURE
shows the curves for the surviving buffer dogs.

TABLE 1 shows that administration of buffer facilitated the defibrillation.
the moment of defibrillation the average arterial *p*H of the buffer dogs was
2, while that of the control dogs was 7.15; mixed venous *p*Hs at this time
e about 0.1 unit lower. It would appear that an initial administration of
./kg. of buffer after cardiac arrest helps retard the developing acidosis, and
unknown reasons facilitates defibrillation.

n these defibrillation experiments,* no other drugs were used, no calcium

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* These experiments were done in collaboration with Katrina T. McArthur.

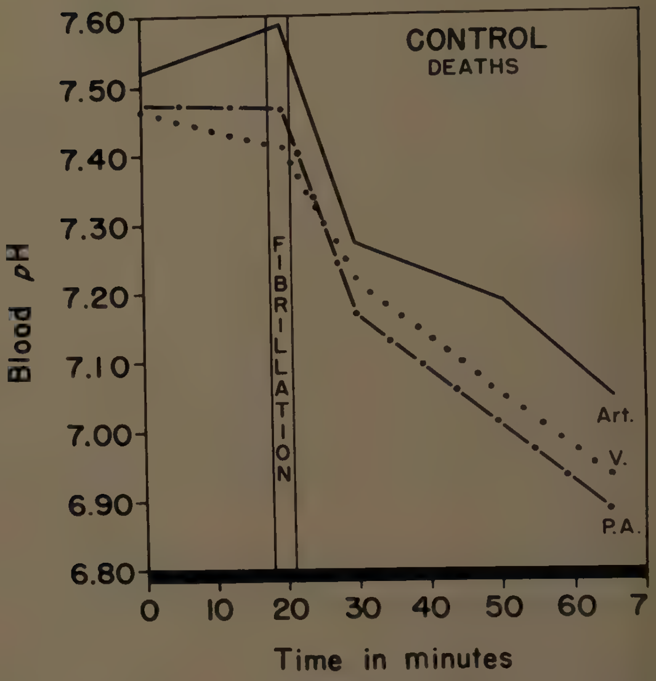

FIGURE 11.

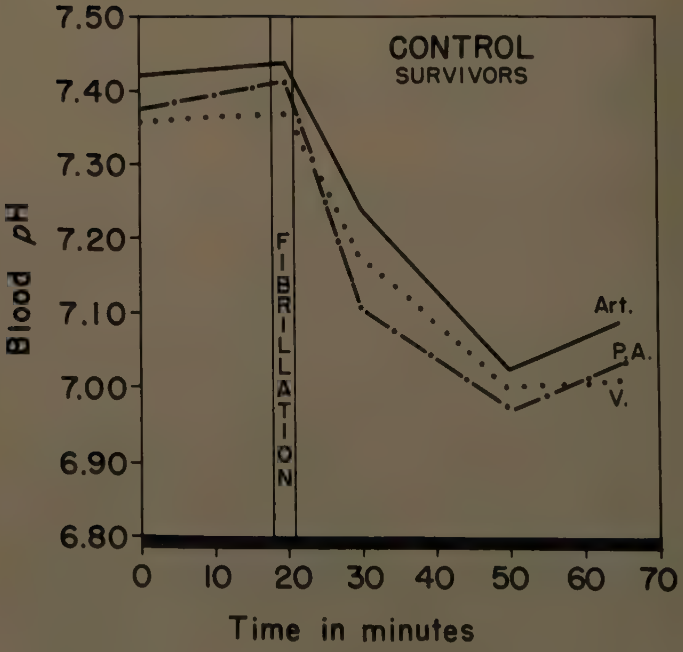

FIGURE 12.

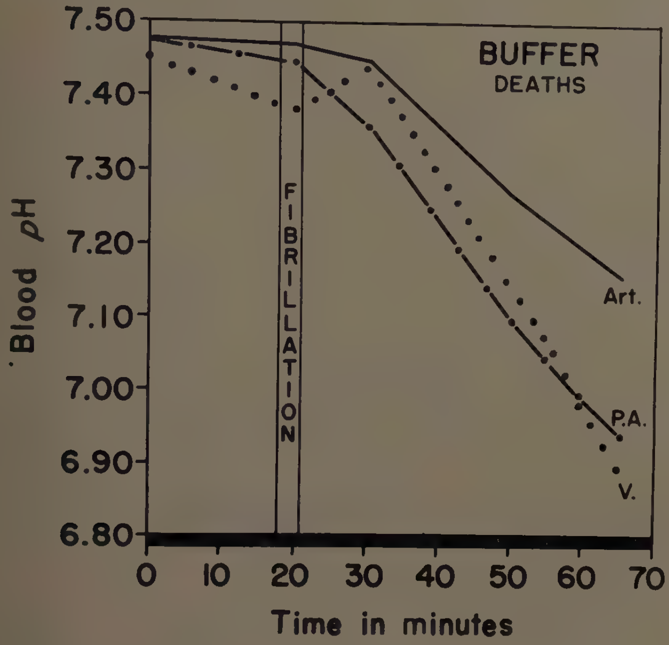

FIGURE 13.

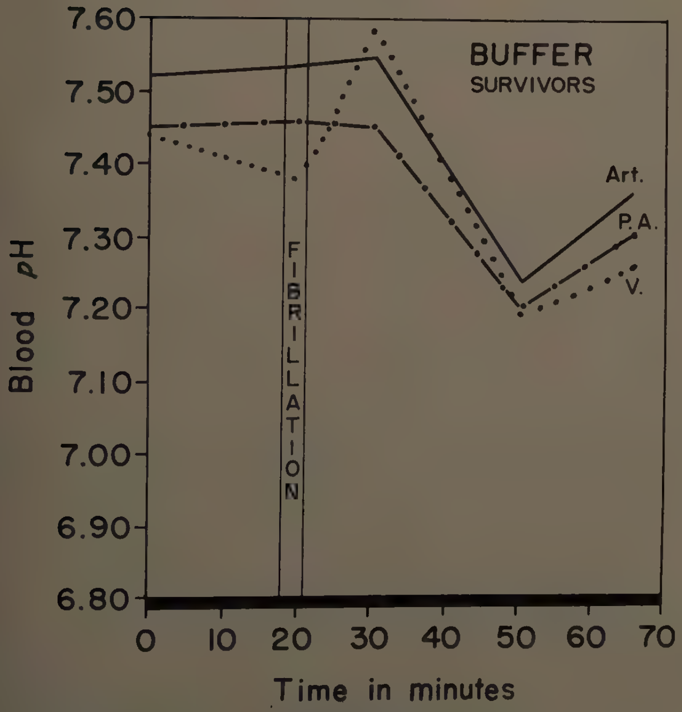

FIGURE 14.

was given, and no attempt was made to readjust the blood *p*H after the sin
dose of buffer. We have no doubt that calcium should be given, and we su
gest that possibly it would be helpful to have calcium added to Tris prepa
tions intended for cardiac resuscitation.

Clinical application. These clinical studies were undertaken with Char
Lyons, Sterling Edwards, Katrina McArthur, Lionel Bargeron, and Mer
Bradley.

Tris buffer has been administered to a number of medical patients w
metabolic acidosis arising from a low cardiac output. Generally, the buf
was administered so as to bring the blood *p*H toward, but not necessarily
to, normal. For example, in three moribund adult patients diagnosed as ha
ing generalized septicemia, and being maintained on pressor agents, about
cc. of buffer administered over not less than two hours was necessary to bri
the *p*H from an average of 7.07 to 7.30. In these patients no clinical impro
ment was noted, but the rate of administration of pressor agents could be
duced. In 10 other patients restoration of responsiveness and lucidity w
observed after giving the buffer. No changes in serum sodium, potassiu
chloride, glucose, or hematocrit were observed. All of these patients w

TABLE 1
EFFECT OF AMINE BUFFER ON DEFIBRILLATION IN DOGS

	N	Defib. on shock No.						Could n defibrill
		1	2	3	4	5	6	
Control	10	3	2	3			1	1
Buffer	11	6	4	1				0

receiving glucose solutions and all were being given oxygen. One patient
37-year-old woman who developed bradycardia and hypotension followin
vena caval ligation, dramatically improved with clearing of the sensorium
her venous *p*H was brought from 7.18 to 7.33 with 500 cc. of Tris buffer o
a 3-hour period. Blood chemistry remained normal during the next 96 hou
except that one *p*H of 7.52 was measured. She is alive and well.

Data concerning *p*H obtained from a study of 13 cardiac arrest patient
shown in TABLE 2. The fall in blood *p*H, observed in the animal experime
is readily apparent here as well. Administration of the initial dose (wh
averaged 5.9 cc./kg.) brought the *p*H from 7.05 to 7.27. The heart was
suscitated in only 5 of these patients, but several of the others were conside
terminal (2 had generalized septicemia) at the time of arrest. One patien
23-year-old neurosurgical patient, had resisted repeated attempts at defibri
tion; after the administration of 600 cc. of buffer he was successfully defib
lated electrically. He made a rapid recovery.

In open-heart surgery the buffer has been administered in the extracorpo
circuit on 4 occasions and postoperatively 7 times. In the first 4 cases it
employed to counteract metabolic acidosis that developed as soon as the t
body perfusion rate was decreased preparatory to returning the patient to
own support. In these cases the surgical correction planned could not

sfactorily completed and the patient was unable to maintain a cardiac out- compatible with survival. These patients were maintained on partial ulation; we periodically stopped the perfusion to determine if their own rts could "take over." During these trial periods of only a few minutes blood pH would drop as much as 0.2 unit; these drops were corrected with fer.

n the other 7 open-heart surgical cases the buffer was given in the post- rative period to compensate for a metabolic acidosis resulting from a low diac output. Two of these patients survived and are well today. In one hese, an adult who had aortic valvulopasty during a 2-hour perfusion while

TABLE 2
CARDIAC ARREST PATIENTS

Patient		Estimated time since onset of arrest (min.)	Blood pH			Tris infused	
Age (years)	Weight (kg.)		Before Tris*	After initial dose	After total dose	Initially (cc.)	Total (cc.)
1.5	11	45	7.04	7.46	7.46	100	100
33	54	5	7.25	7.37	7.37	200	200
4	18	60+	6.86	7.10	7.29	200	500
23	58	45	—	7.39	7.39	600	600
40	64	25	6.98	7.49	7.49	600	600
44	48	60	7.19	7.40	7.44	400	900
3	15	5	7.05	7.11	7.44	60	480
54	53	1	6.97†	7.58	7.75‡	250	550
47	68	2	7.27	7.28	7.43	100	175
32	58	5	7.05	6.97	6.97	300	700
40	63	5	—	7.28	7.28	300	300
47	58	2	6.96	7.01	7.25	200	800
22	68	5	7.03	7.08	7.06	400	4000§
rages 30	48	20	7.05	7.27	7.34	285	761

This column indicates samples taken after the time shown. The doses are of Abbott oratories' 0.3 *M* THAM, containing NaCl and KCl.

This patient had received 50 cc. of Tris before the first pH was taken.

All samples were taken from the right atrium or vena cava; they probably contained e buffer than did the mixed venous blood.

Administered over a 48-hour period.

coronary arteries were perfused at about 300 cc./min., administration of buffer restored his blood pressure coincident with an increase in blood pH. the same time his Levophed requirement decreased, although it was 9 days ore the intravenous infusion of this pressor agent could be entirely stopped. second patient, a 6-year-old girl operated upon for ostium primum during -hour period of total by-pass and an additional hour of partial perfusion, eloped an acidosis postoperatively. During the entire period of perfusion 25° C.) her arterial pH remained normal (actual reading 7.56 at 25° C.), arterial CO_2 was around 36 volume per cent, and the venous saturation raged 85.3 per cent (78.4 to 94.0). One and one-half hours after perfusion arterial pH was only 7.16, and the pulmonary artery saturation ranged und 45 per cent: a level we regard as very dangerous. After 50 cc. of Tris pH was 7.26; after a total of 100 cc. was given, it was 7.26; after 250 cc., the pH was 7.33; and after 350 cc., it was 7.42. The buffer was given ove 5-hour period. The venous saturation climbed to 70 per cent during this ti Interestingly, in this patient the mean arterial pressure was satisfactory mm. mean) and constant throughout, and provided no clue as to her precari situation. After this correction of her cardiac output, she continued to do v well.

Aside from the salicylate case already reported,[10] the buffer has been u in the treatment of two infants. One two-months'-old infant developed bra cardia and had ineffectual contractions following creation of an auricu septal defect (to correct severe cyanosis caused by a single ventricle and mi atresia). Calcium chloride and massage did not improve the condition. I lowing the administration of 20 cc. of Tris, the heart rate increased and c tractions became vigorous. Venous pH increased from 7.02 to 7.26, p changed from 55 to 25 mm., and standard bicarbonate from 11.5 to 15.0. hour later, following the administration of 5 cc. more (in 50 cc. of 5 per glucose), the venous pH was 7.26, pCO_2 42, and standard bicarbonate 18. other case was that of a premature infant with pneumonia, who was give cc. of Tris, one half in 4 hours, and the remainder over the next 12 hours. venous pH increased from 6.84 to 7.24 and she continually improved. T weeks after this procedure she is gaining weight and appears to be on the to recovery.

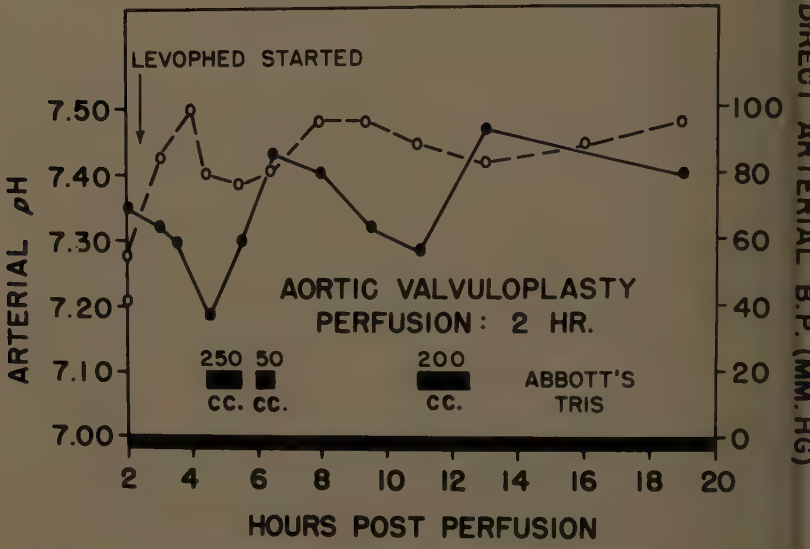

FIGURE 15.

Summary

Tris is useful in correction of metabolic acidosis when this results fro condition that is thought to be reversible and in which prolonged exposur a low blood pH may cause further damage. For example, it may be us in treating postoperative acidosis in open-heart surgery patients in who cardiac output immediately postoperatively may be caused by unavoidable umatic or hypoxic damage to the myocardium. It may be useful in coun- cting the acidosis that sometimes follows moderate hypothermia or circu- ry arrest in deep hypothermia. It may be useful in correcting metabolic dosis in patients in chronic heart failure undergoing aortic or mitral valve gery, but in whom the administration of sodium may be considered un- rable.

Tris may be useful where an alkaline “indicator solution” is required for gnostic purposes.

Depression of respiration and a decrease in brain oxygen availability are un- rable effects. Patients should be well ventilated while receiving this amine fer, which should be administered only when blood *p*H and carbon dioxide els can be frequently measured.

ince 2-amino-2-hydroxymethyl-1,3-propanediol facilitates defibrillation, entiates pressor drug activity, and counteracts acidemia, it may be useful he treatment of cardiac arrest. For this purpose, an initial dose of 5 cc./kg. *M*), preferably with added calcium, can be given.

Blood *p*H is not a satisfactory criterion for judging Tris dosage but, of course, hould be measured. Perhaps blood amine levels will be useful. Intra- ular buffering is a new approach and will require new ways of thinking and methods of measurement. In general, I think doses have been too high ause we were still thinking in terms of returning blood *p*H to normal. It be that blood levels of 2 mEq./l., or lower, will provide physiologically ificant buffering.

Acknowledgment

am indebted to Samuel J. Anderson of Abbott Laboratories for providing erous supplies of that firm's sterile antiacidosis preparation containing 36.3 tris(hydroxymethyl)ammomethane, 1.75 gm. sodium chloride, and 0.37 potassium chloride when diluted to 1 l.

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EFFECTS OF 2-AMINO-2-HYDROXYMETHYL-1,3-PROPANEDIO ON ACID-BASE BALANCE DURING TEMPORARY THORACIC OCCLUSION OF THE INFERIOR VENA CAVA IN THE DOG

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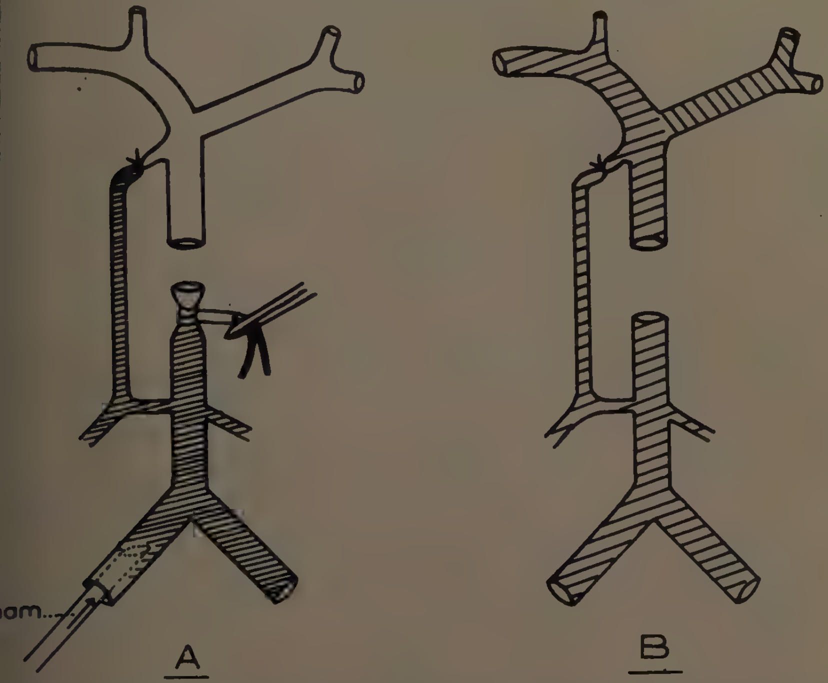
Introduction

The purpose of this work was to test the buffering capacity of tris(hydro methyl)aminomethane (THAM)[1,2] during a type of severe acidosis encounte clinically. This acidosis was produced by temporary occlusion of the thora portion of the inferior vena cava, a complication appearing sometimes dur hepatic lobectomy or open heart surgery with extracorporeal circulation. D ing hepatic resections the suprahepatic vein may be so short that a clamp the inferior vena cava is needed. During open heart procedures the cann in the inferior vena cava may be of too small a caliber or kinked or too dee inserted into the suprahepatic vein resulting in incomplete drainage of the bl from the lower part of the body.

Marked acidosis develops in the lower part of the body during the time occlusion of the inferior vena cava (FIGURE 1). The collateral circulation tween the lower and upper part of the body through the azygos and the ca caval system is inoperative during an extracorporeal circulation because azygos vein is ligated. When the occlusion on the inferior vena cava is releas acidotic blood is sent into the systemic circulation where it mixes. An acid consequently is superimposed upon the venous hypertension and ischemia the lower part of the body. In addition, during extracorporeal circulation venous return to the pump is diminished, there will be an inadequate p fusion and further acidosis. Perfusions longer than 60 min. and eleva coronary flow return to the pump are additional possible sources of acido Therefore a drug that could buffer acidotic blood during open heart surg may be useful. The temporary occlusion of the thoracic portion of the ferior vena cava was used to illustrate acidotic complications that deve clinically, and THAM was administered to correct the acidosis.

Material and Methods

Dogs anesthetized with sodium pentothal were intubated and ventila mechanically with 100 per cent oxygen. The femoral artery and vein w cannulated for arterial and venous blood-pressure monitoring and withdra of blood specimens. The venous catheter was inserted high in the iliac ve Heparin was administered at the rate of 1 mg. 5/kg. to prevent clotting arou the catheter. Through a right lateral thoracotomy a tape was placed arou the inferior vena cava above the diaphragm. The azygos vein was ligate order to prevent any major collateral circulation from the inferior vena c to the upper part of the body. To ascertain the tolerance to the occlusion the inferior vena cava, a series of control experiments was done compar the length of occlusion time with the survival rate and the rate of appeara of symptoms of medullar ischemia and venous abdominal-hypertension, s araplegia and bloody stools. In these control dogs, occlusion of the in- or vena cava was maintained during 15, 25, 30, and 35 min. after ligation he azygos vein. The respiration was continuously controlled but no drug given. After release of the occlusion, acidotic blood from the lower part he body was mixed into the systemic circulation. Blood samples were ined from the inferior vena cava, superior vena cava, and the femoral ry for pH and total CO_2 measurements. These measurements were done a glass electrode thermostated at 37.5° C. and a Radiometer No. 4 pH er and a Van Slyke manometer. At the end of the procedure the thora-



IGURE 1. Schematic description of experimental procedure. (A) Inferior vena cava sion and simultaneous administration of THAM; (B) relief of the occlusion and mixing e blood from the lower and upper parts of the body.

my was closed, a drain left in place, suction applied, and the dog left to ver.

a second series of experiments, the temporary occlusion of the inferior a cava was followed by a perfusion through an iliac vein of a 0.3 M solu- of THAM at the rate of 1 ml./kg./min. Thirty mEq. of NaCl were added ach liter of solution. This perfusion was started at different intervals wing occlusion, or just after the release of the tape.

Results and Discussion

this study, attention was primarily focused upon the acute consequences nferior vena cava occlusion. The later complications of this procedure, as paraplegia, are also reported.

Control dogs. Four dogs were used in this series. The occlusion time respectively 15, 25, 30, and 35 min. The first dog survived in good conditi All other dogs had paraplegia and bloody stools after the operation. second and third animals survived, and the paraplegia disappeared after 6 7 days. The fourth dog died, still paraplegic, on the sixth day. TABLE and 2 illustrate the experiments performed on the last 2 control dogs. Th experiments indicate that inferior vena cava occlusion produces a severe acid in which there is a marked CO_2 retention and a fall in bicarbonate. Moreo

TABLE 1

Period	Sample	*p*H	Total CO_2 Vol %	Total CO_2 m*M*/l.	*p*CO_2 mm Hg.	HCO m*M*/
Before occlusion	Venous Arterial	7.25 7.24	61.4 57.9	27.6 26.0	60.6 58.4	25. 24.
Eight min. after occlusion	S.V.C.* I.V.C.†	7.04 6.90	63.4 66.8	28.5 30.0	97.4 136.4	25. 25.
Ten min. after releasing occlusion	Venous Arterial	7.00 7.05	59.8 56.4	26.9 25.3	99.8 84.9	23. 22.

* Superior vena cava.
† Inferior vena cava.

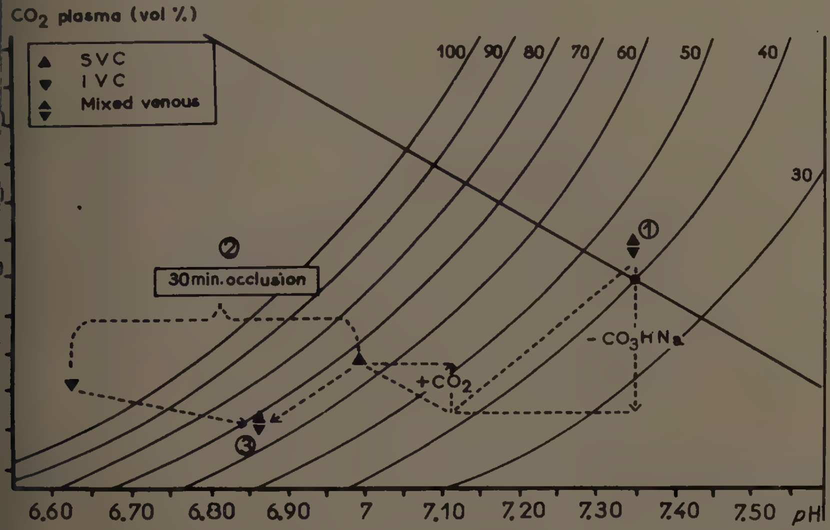
TABLE 2

Period	Sample	*p*H	Total CO_2 Vol. %	Total CO_2 m*M*/l.	*p*CO_2 mm Hg.	HCO m*M*/
Before occlusion	Venous Arterial	7.36 7.33	54.1 52.1	24.3 23.4	42.0 43.2	23. 22.
Thirty min. after occlusion	S.V.C.* I.V.C.†	7.00 6.64	39.0 36.4	17.5 16.4	65.1 121.6	15. 12.
Ten min. after releasing occlusion	I.V.C.† Arterial	6.87 6.92	31.5 30.7	14.1 13.8	68.2 60.2	12. 12.

* Superior vena cava.
† Inferior vena cava.

in spite of the occlusions of the inferior vena cava and azygos vein, there is important admixture of blood from the inferior and superior venae cavae ducing a generalized acidosis in the whole body (FIGURE 2).

Dogs treated with THAM. The second series of experiments was perfor on 9 dogs. In 2 experiments, both superior and inferior venae cavae simultaneously clamped and a solution of 0.3 *M* THAM intravenously ministered at the start of the occlusion. One dog survived 10 min. of occlus of the superior vena cava and 11 min. of occlusion of the inferior vena ca but remained permanently paraplegic. A second dog died of ventric fibrillation after 14 min. of occlusion of the superior vena cava and 11 min usion of the inferior vena cava. In 6 other dogs the inferior vena cava was uded for varying periods of time, from 30 to 44 min. THAM was adistered as described previously. These animals survived but were temrily paraplegic for 2 to 4 days.



IGURE 2. Diagram indicating the changes in acid-base balance in the blood of superior inferior venae cavae and mixed venous blood of a dog undergoing 30 min. of occlusion of nferior vena cava.

TABLE 3

Period	Sample	*p*H	Total CO_2		*p*CO_2 mm. Hg.	HCO_3^- m*M*/l.
			Vol. %	m*M*/l.		
re occlusion	Venous	7.28	62.6	28.1	57.9	26.4
	Arterial	7.31	56.9	25.6	49.3	24.1
ty min. after oc- usion and 5 min. THAM perfusion	S.V.C.*	7.51	58.4	26.2	32.6	25.2
	I.V.C.†	6.92	58.0	26.0	113.7	22.6
min. after releas- g occlusion and ntinuous THAM fusion	Venous	7.27	65.4	29.4	61.8	27.5
	Arterial	7.26	62.0	27.8	59.9	26.0

Superior vena cava.
Inferior vena cava.

ABLES 3 and 4 illustrate the results of 2 of these experiments. Ten minutes r release of the occlusion and after 15 min. of THAM perfusion (TABLE 3) -base balance of venous and arterial blood is close to control. TABLE 4 cates that THAM administration produces a marked alkalosis in the upper of the body, while correcting the acidosis in the lower part (FIGURE 3).

Table 4

Period	Sample	pH	Total CO$_2$		pCO$_2$ mm. Hg.	HCO$_3$ mM/l
			Vol. %	mM/l.		
Before occlusion	Venous	7.33	58.5	26.3	48.6	24.8
Thirty min. after occlusion	S.V.C.*	6.78	54.8	24.6	141.2	20.4
Twenty-seven min. after occlusion	I.V.C.†	7.04	49.6	22.3	76.2	20.0
Forty min. after occlusion and 10 min. of THAM perfusion	S.V.C. I.V.C.	7.44 7.90	75.9 55.6	34.1 25.0	49.5 12.9	32.6 24.6
Ten min. after releasing occlusion	Venous	7.18	48.6	21.8	55.7	20.2

* Superior vena cava.
† Inferior vena cava.

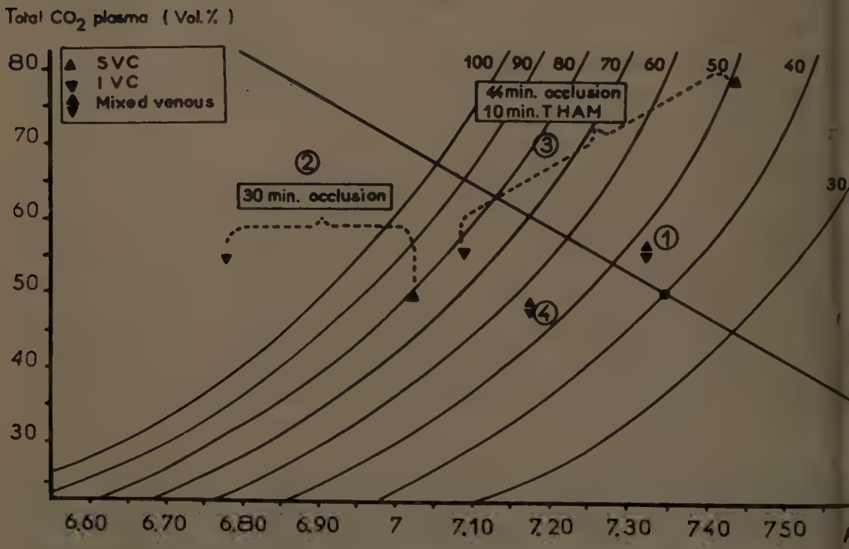


Figure 3. Diagram indicating the changes in acid-base balance in the blood of supe and inferior venae cavae and mixed venous blood in a dog undergoing 44 min. of occlusio the inferior vena cava. The last 10 min. of occlusion were accompanied by administrat of THAM.*

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* We are aware of the fact that this diagram derived from Davenport is not absolutely curate when THAM is perfused, but it gives a good demonstration of the acid-base balan

Conclusions

n dogs, maintained on controlled respiration with 100 per cent oxygen, 30 . occlusion of the inferior vena cava produces a severe acidosis in the lower t of the body with CO_2 retention and bicarbonate dissociation. Though severe, a similar acidosis appears also in the upper part of the body in spite he ligation of the azygos vein. After relief of the occlusion, the dog is still lotic. The degree of this acidosis is a function of the acidosis that had viously prevailed in the 2 halves of the body. In a second series, occlusion he inferior vena cava was performed in the same way, but THAM was ninistered at the rate of 1 ml./kg./min. Within 10 min., the blood in the rior vena cava reduces its acidity by half and the blood in the superior vena a becomes alkaline. This procedure allows for a rapid correction of the losis following unclamping of the inferior vena cava.

hese experiments indicate that THAM may be useful in correcting the losis produced by temporary occlusion of the thoracic portion of the inor vena cava.

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EFFECTS OF 2-AMINO-2-HYDROXYMETHYL-1,3-PROPANEDIO ON OXYGEN TOXICITY IN MICE*

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The use of high oxygen tensions in man is accompanied by toxic effects. though these effects can be postponed by a number of compounds, for insta central-nervous-system depressant drugs such as the barbiturates,[1] or a sympathomimetic agents such as chlorpromazine,† the etiology of oxygen to ity is not fully understood. There has been much discussion as to the p if any, played by carbon dioxide in its causation.

It seemed therefore of interest to study the effect on oxygen toxicity of b ering agents that may modify storage and transport of carbon dioxide maintain acid-base balance of the body within normal limits in the prese of a respiratory or a metabolic acidosis.

Two compounds were selected in this study, the commonly used inorga buffer, sodium bicarbonate, and an amine buffer of low toxicity, 2-amin hydroxymethyl-1,3-propanediol, also called tris(hydroxymethyl)aminometha or Tris, or THAM.[2]

In man, the first manifestations of oxygen toxicity are convulsions indicat central-nervous-system impairment; with continued exposure, lung dam occurs. In the smaller animals the picture is complicated by the earlier occ rence of pulmonary damage. The relative prominence of central nervous pulmonary effects depends to some extent upon the particular conditions the exposure to high oxygen tension.

The experiments described in this paper were carried out on mice breath 100 per cent oxygen at gauge pressures of 30, 42, and 55 lbs. per square i (psi), that is, 3, 3.8, and 4.7 atmospheres absolute. Toxicity was measured the duration of exposure before the onset of convulsions, and by the survi over a 24-hour period following exposure for a fixed time. Measureme were also made of the oxygen uptake of mice breathing 100 per cent oxygen atmospheric pressure. The substances tested, THAM and NaHCO$_3$, w used in a 0.3 molar concentration. The controls were 0.3 *M* THAM buffe to *p*H 7.4 with HCl (which decreases the buffering capacity of the amine 75 per cent), and isotonic saline.

Methods

HIGH PRESSURE EXPERIMENTS

Webster Swiss mice of approximately 30 gm. were used. It was establis by a series of preliminary observations that no apparent harm resulted f intraperitoneal injections of the test substances, administered 3 times d

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* The investigation reported in this paper was supported by Research Grant NsG-6 from the N.A.S.A. and Research Grant C-4502(C) from the National Cancer Institute, lic Health Service, Bethesda, Md.

† W. M. D. Paton. 1955. Personal communication.

3 weeks. Two series of experiments were carried out, the conditions of pression differing slightly in each.

ries I: groups of 10 mice of the same sex were used for each experiment. mice were injected with one substance and 5 with another, the injections isting of 1 ml. of 0.3 M THAM, 1 ml. of 0.3 M $NaHCO_3$, 1 ml. of 0.3 M M buffered to pH 7.4 with HCl or 1 ml. isotonic NaCl. The mice were placed in the compartments of a specially constructed pressure chamber URE 1), which was flushed with 100 per cent oxygen for 5 min. and then ght to the appropriate pressure in 3 min. The time from the injection full pressure was reached was as close as possible to 30 min. The con- ion time was taken in minutes from the attainment of full pressure until onset of repeated involuntary and apparently purposeless muscle twitchings ne front legs. This sign was usually preceded by a period of "washing" ements, but could be easily distinguished from them.

ries II: the details differed from Series I in the following ways:

) Each group compressed consisted of 9 mice, 3 of which were injected THAM, 3 with $NaHCO_3$, and 3 with saline. The volume of the injec- s was adjusted on the basis of weight, 1 cc. being given to 30 gm. mice, proportionate amounts for others.

) To allow time for absorption and distribution of the drugs the mice were n 3 injections, 6 hours, 3 hours and 20 min. before compression.

) Compression was carried out at a constant rate instead of in a constant , 7 min. being allowed to reach 30 psi, 10 min. for 42 psi and 15 min. for 55

) Slightly different end points were used, generalized muscle spasms being in the experiments at 42 and 55 psi, and twitching of the cervical muscles) psi. This inconsistency means that results at different pressures are not tly comparable with each other; the reason for it was to limit compression) psi to 45 min. as part of the 24 survival experiments that will be de- ed later.

Twenty-Four Hour Survival Time

llowing the observation of convulsion time in Series II, pressure was tained until the mice had been exposed to full pressure for a total of 45 at 30 or 42 psi or 16 min. at 55 psi. The mice were then returned to their s, and allowed food and water ad lib. The number of survivors was ted 24 hours later.

Oxygen Uptake Experiments

oups of 10 mice of one sex were given 1 ml. of the test solution intraperi- ally and, 15 min. later, were placed in the chamber, which was flushed with er cent oxygen for 5 min., closed (at atmospheric pressure), and connected a spirometer containing 100 per cent oxygen. The oxygen consumption measured over a period of 1 hour. Six experiments were conducted using al saline injection, 4 using THAM, 4 using $NaHCO_3$, and 3 using buffered 7.4) THAM.

e results in each group were averaged and reduced to STPD and divided

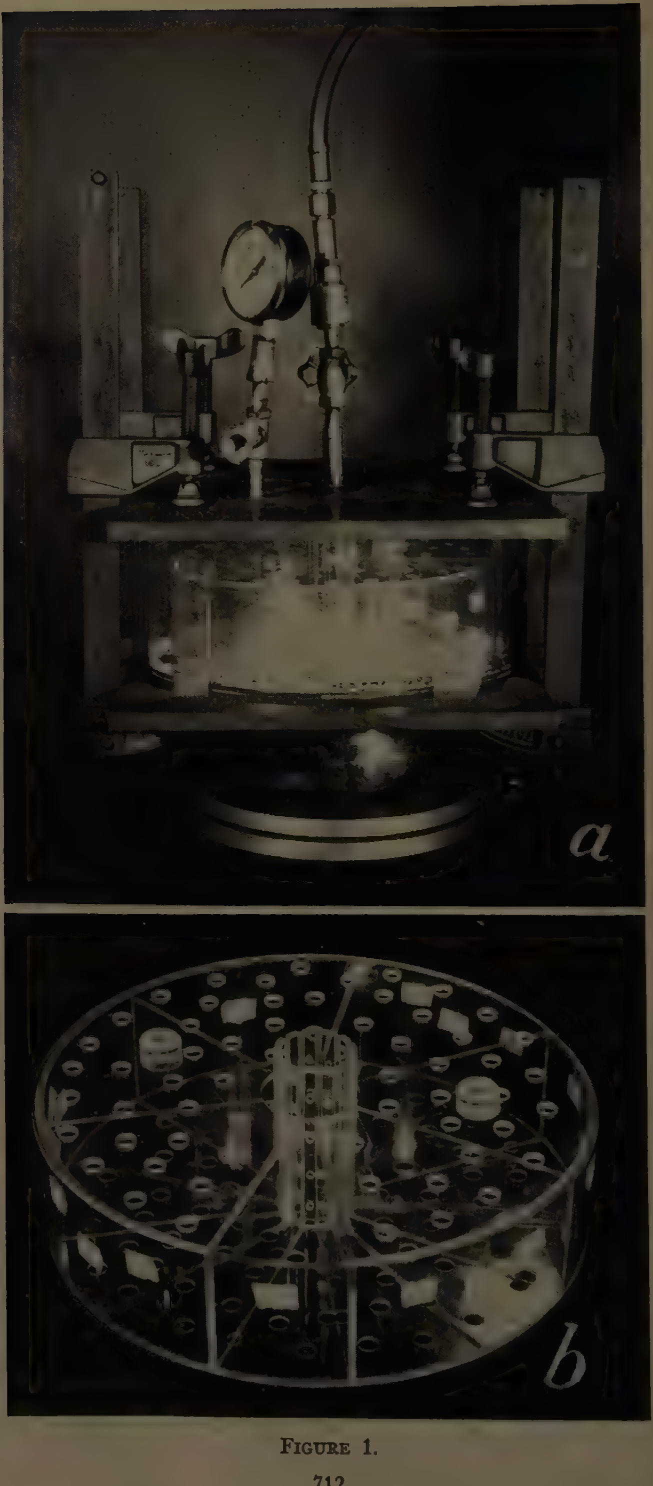


FIGURE 1.

e total weight of the mice to give the oxygen consumption per gram of weight per hour.

Results

HIGH-PRESSURE EXPERIMENTS

Series I

irty psi. The convulsion times are given in TABLE 1. Experiments 7, d 9 contain only 1 group of 5 mice; the others had been injected with 0.2

TABLE 1
SERIES I, 30 p.s.i.: CONVULSION TIMES (MINUTES)

i-	Saline	THAM	$NaHCO_3$	Buffered THAM
	27, 30, 30, 31, 40	42, 46, 64, 67, 67		
		48, 49, 50, 53, 59	18, 18, 19, 20, 25	
	28, 29, 30, 31, 32	64, 65, 68, 64, 72		
		49, 52, 58, 58, 61		17, 18, 28, 32, 50
		74, 74, 82, 82, 83		42, 43, 44, 49, 57
		39, 47, 51, 51, 52	20, 20, 21, 22, 23	
		54, 56, 56, 61		
		66, 66, 68, 69, 71		
			17, 18, 19, 20, 25	
	26, 28, 30, 30, 31, 31, 32, 33, 33, 34			
				37, 38, 39, 43, 44, 44, 45, 45, 46, 47,

TABLE 2
s I, 30 p.s.i.: CONVULSION TIMES FOR ALL EXPERIMENTS, GROUPED AND ARRANGED IN ASCENDING ORDER

(20 mice)	26, 27, 28, 28, 29, 30, 30, 30, 30, 31, 31, 31, 31, 32, 32, 33, 33, 34, 37, 40
(39)	39, 42, 46, 47, 48, 49, 49, 50, 51, 51, 52, 52, 53, 54, 56, 56, 58, 58, 59, 61, 61, 64, 64, 65, 66, 66, 67, 68, 68, 69, 69, 71, 72, 74, 74, 77, 82, 82, 83
O_3 (15)	17, 18, 18, 18, 19, 19, 20, 20, 20, 20, 21, 22, 23, 25, 25
ed THAM (20)	17, 18, 28, 32, 34, 37, 38, 42, 43, 43, 44, 44, 44, 45, 45, 46, 47, 49, 50, 57

a_2CO_3 but convulsed before beginning pressurization, and the study of ubstance was subsequently abandoned for this reason. The convulsion for all experiments at each pressure were then grouped and arranged in of magnitude, as listed in TABLE 2. From this table the cumulative fre-

URE 1. The pressure chamber (*a*) consists of a transparent plastic cylinder (½ inch 12 inches O.D., 4½ inches high) held between 14-inch × ½-inch steel plates. The te incorporates a centrally placed male Schraeder oxygen inlet, a pressure gauge and a tlet, and can be rapidly placed or removed by means of 4 quick-release clamps. The re seal is maintained by O-rings at top and bottom. Inside the chamber a transparent ted plastic holder (*b*) enables as many as 9 mice to be observed in individual compart- separated by movable partitions. Soda lime may be placed beneath the floor of the and in the central core. The whole apparatus is mounted on a turntable and may be d to facilitate observation.

quency distribution curves of convulsion times were plotted, using a logarith time scale (FIGURE 2). The 50 per cent, or median, convulsion times to nearest half minute, as determined from the table and curves were:

Saline	31 min.
THAM	61.5 min.
$NaHCO_3$	20.5 min.
Buffered THAM	43.5 min.

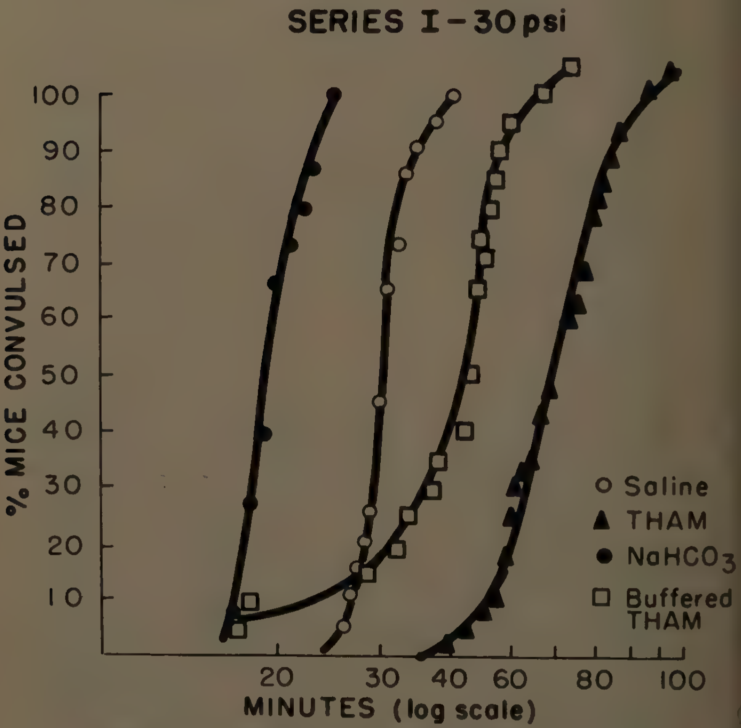


FIGURE 2. Cumulative convulsion times, Series I; 30 psi.

Forty-two psi. The convulsion times are given in TABLE 3. (The ta showing the times grouped and arranged in order of magnitude for this the following experiments are omitted to save space; they can be deduced f TABLE 3 and are shown graphically in FIGURE 3, which combines all the res of Series I.) The cumulative curves are shown in FIGURE 4. The 50 per convulsion times were:

Saline	3.5 min.
THAM	17 min.
$NaHCO_3$	11 min.
Buffered THAM	6 min.

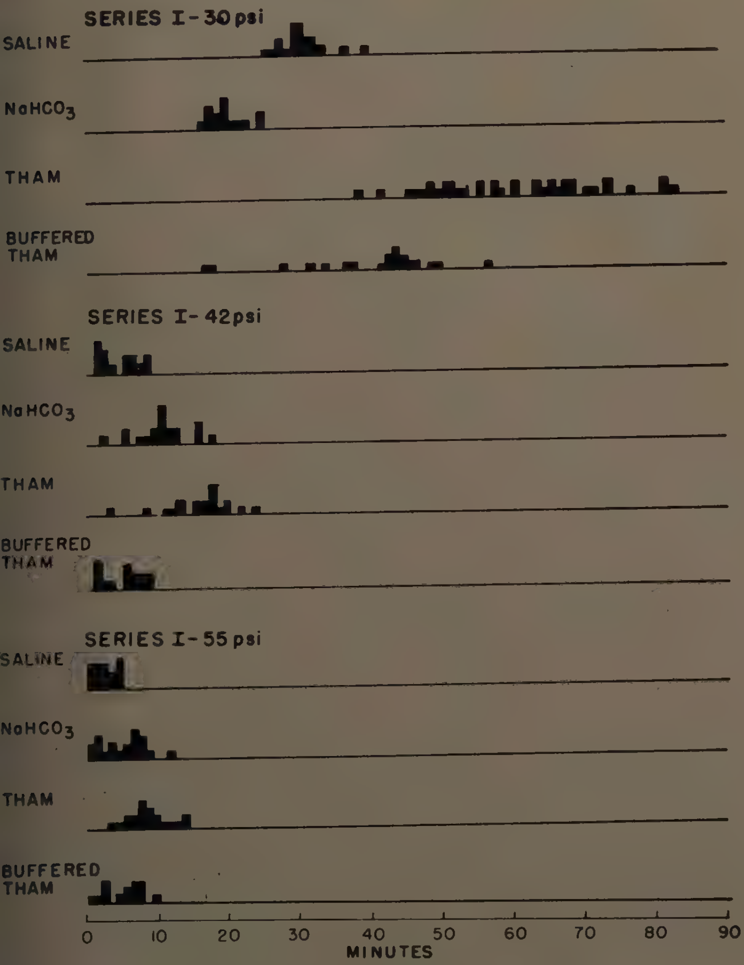
It will be noted that there is a considerable shortening of the latent pe

TABLE 3

SERIES I, 42 p.s.i.: CONVULSION TIMES (MINUTES)

ment	Saline	THAM	NaHCO₃	Buffered THAM
1	2, 2, 2, 3, 3	4, 13, 13, 14, 20		
2	2, 4, 6, 9, 9	12, 17, 18, 18, 19		
3	3, 6, 7, 7, 8	16, 18, 18, 20, 24		
4			7, 7, 10, 11, 11	
5			12, 13, 16, 16, 18	
6			3, 8, 9, 10, 11, 11, 11, 12, 13, 16	
7		9, 14, 16, 17, 22		2, 2, 6, 7, 8
8				2, 2, 3, 4, 6, 6, 7, 8, 9, 9

ther groups of mice injected with 0.2 M Na_2CO_3.



URE 3. Convulsion times, Series I; all pressures. Each small square represents one

for all substances, and that the curve for $NaHCO_3$ now lies between that saline and that for THAM.

Fifty-five psi. The convulsion times are given in TABLE 4, the grouped res at 55 psi appear in FIGURE 3, and the cumulative curves in FIGURE 5.

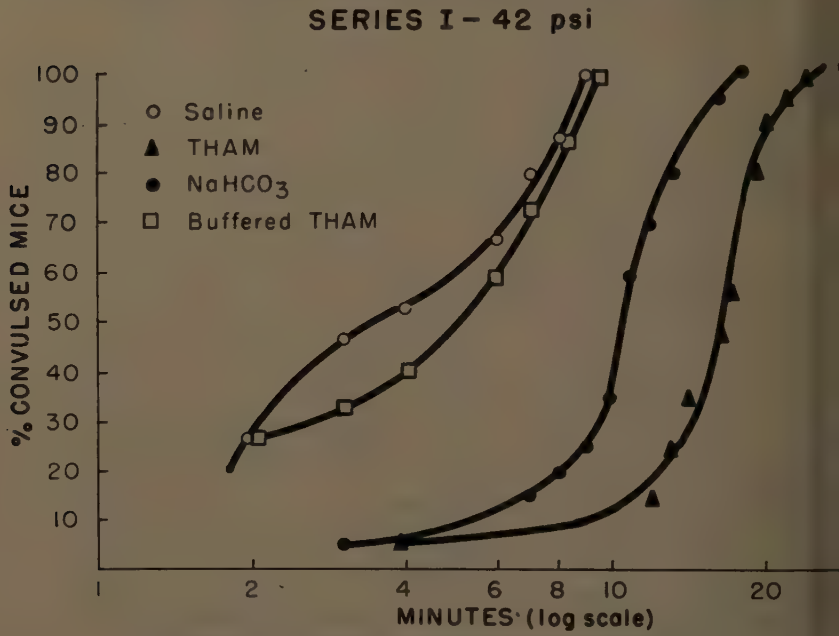


FIGURE 4. Cumulative convulsion times, Series I; 42 psi.

TABLE 4

SERIES I, 55 P.S.I.: CONVULSION TIMES (MINUTES)

Experiment	Saline	THAM	$NaHCO_3$	Buffered THA
1	1, 1, 2, 3, 4	4, 6, 8, 9, 9		
2	1, 2, 3, 5, 5	6, 7, 10, 12, 13		
3	2, 3, 4, 5, 5	7, 9, 10, 11, 14		
4			1, 2, 2, 3, 4, 7, 7, 8, 9, 12	
5			1, 2, 4, 5, 6, 6, 7, 7, 8, 8	
6				1, 3, 3, 5, 6, 6 8, 8, 10
7		6, 9, 9, 10, 15		2, 3, 7, 7, 8

A further shortening of the preconvulsive time is shown for all injecti the relative position of the saline and THAM curves being maintained. 50 per cent convulsion times are:

Saline	3 min.
THAM	8.5 min.
$NaHCO_3$	6 min.
Buffered THAM	6 min.

Series II

irty psi. The convulsion times are listed in TABLE 5, graphically shown der of magnitude in FIGURE 6 and the curves represented in FIGURE 7.

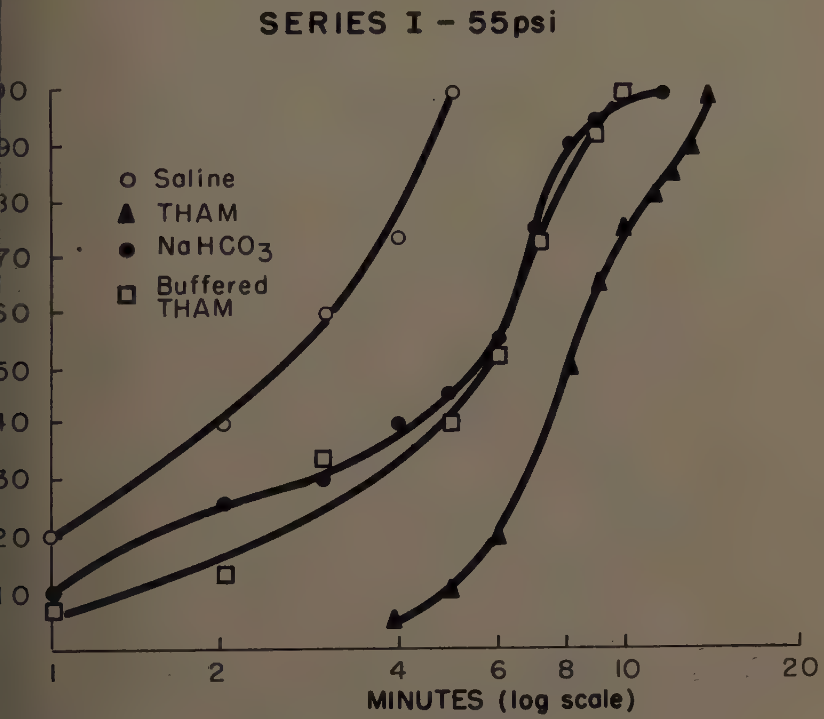


FIGURE 5. Cumulative convulsion times, Series I; 55 psi.

TABLE 5
SERIES II, 30 p.s.i.: CONVULSION TIMES (MINUTES)

xperiment	Saline	NaHCO₃	THAM
16	5, 7, 9	20, 25, 31	11, 12, 13
17	5, 6, 7	13, 16, 25	14, 30, 32
18	6, 7, 7	15, 18, 20	13, 14, 14
19	8, 11, 12	40, 43, 51	33, 35, 39
21	3, 4, 5	6, 10, 12	20, 21, 22
22	4, 5, 7	12, 14, 15	16, 18, 19

wide variation between experiments 19 and 21 should be noted, but there ready explanation for this. Experiment 20 was abandoned because of a n the pressure seal. The 50 per cent convulsion times are:

Saline	6.5 min.
NaHCO₃	17 min.
THAM	18.5 min.

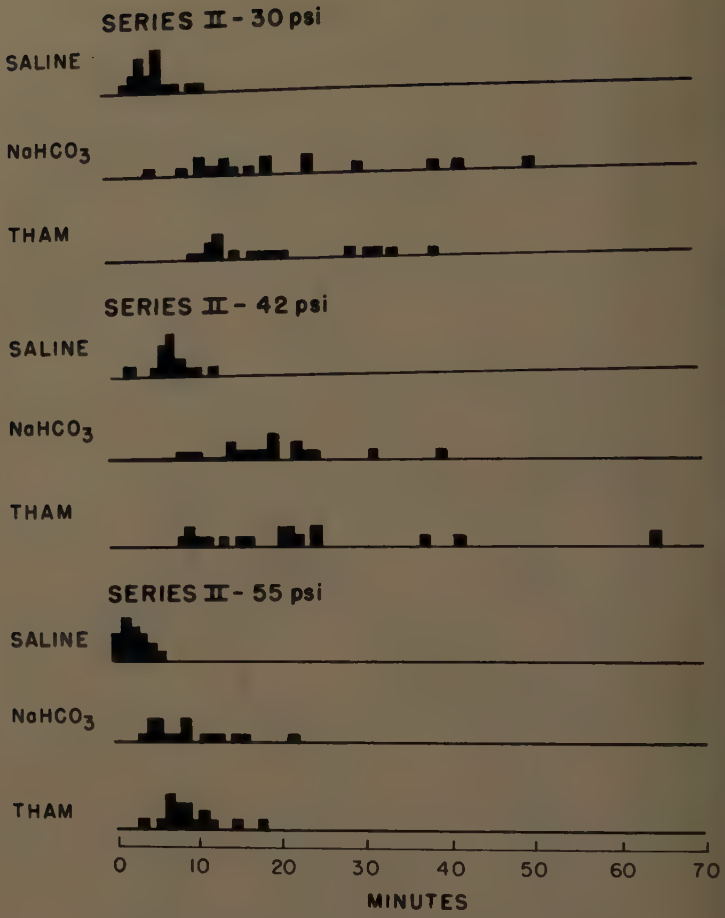


FIGURE 6. Convulsion times, Series II; all pressures.

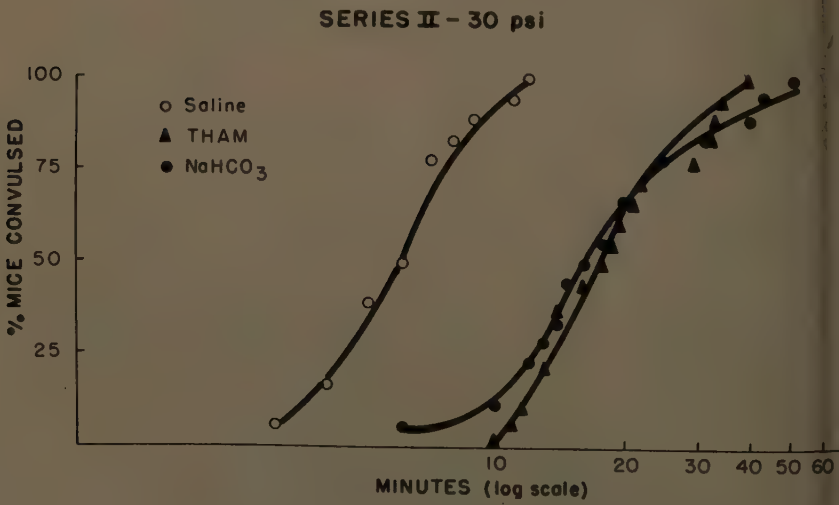


FIGURE 7. Cumulative convulsion times, Series II; 30 psi.

rty-two psi. The convulsion times are given in TABLE 6, grouped and ged in FIGURE 6 and the cumulative curves in FIGURE 8. The 50 per convulsion times were:

Saline	8 min.
$NaHCO_3$	19.5 min.
THAM	21 min.

TABLE 6

SERIES II, 42 p.s.i.: CONVULSION TIMES (MINUTES)

xperiment	Saline	$NaHCO_3$	THAM
10	7, 8, 9	10, 23, 32	16, 22, 42
11	6, 8, 13	23, 24, 39	17, 21, 65
12	5, 7, 8	11, 16, 25	22, 25, 38
13	7, 8, 8	9, 18, 20	10 12, 14
14	9, 10, 11	15, 19, 20	21, 23, 25
15	3, 5, 7	15, 17, 20	9, 10, 11

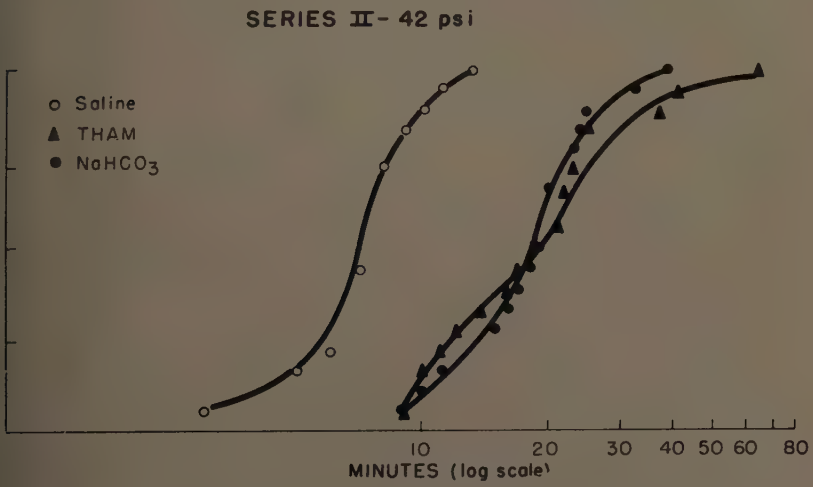


FIGURE 8. Cumulative convulsion times, Series II; 42 psi.

ty-five psi. The results are shown in TABLE 7 and FIGURES 6 and 9. The r cent convulsion times were:

Saline	3 min.
$NaHCO_3$	8.5 min.
THAM	8.5 min.

TWENTY-FOUR HOUR SURVIVALS

e number of surviving mice at 24 hours after the experiments in Series shown in TABLE 8. It will be seen that both THAM and $NaHCO_3$ given r the conditions of this experiment afford a considerable degree of protecgainst the total effects of exposure to high tensions of oxygen.

OXYGEN UPTAKE EXPERIMENTS

The control mice, injected with saline, consumed 2.29 cc./gm./hour oxygen. Those injected with THAM consumed 1.68 cc./gm./hour, NaHCO$_3$ 1.86 cc./gm./hour and, with buffered THAM, 2.27 cc./gm./h Taking the figures for saline as 100 per cent, the figures for THAM, NaHC and buffered THAM represent 73 per cent, 81 per cent, and 99 per cent spectively. Thus there is a marked reduction in oxygen consumption in mice receiving THAM or NaHCO$_3$.

TABLE 7
SERIES II, 55 p.s.i.: CONVULSION TIMES (MINUTES)

Experiment	Saline	NaHCO$_3$	THAM
23	4, 5, 6	13, 15, 16	8, 9, 11
24	1, 2, 3	4, 5, 5	7, 7, 12
25	2, 3, 4	5, 6, 9	7, 8, 15
26	2, 4, 5	6, 8, 9	7, 10, 11
27	1, 2, 3	6, 7, 9	4, 8, 18
28	1, 2, 3	11, 12, 22	6, 9, 10

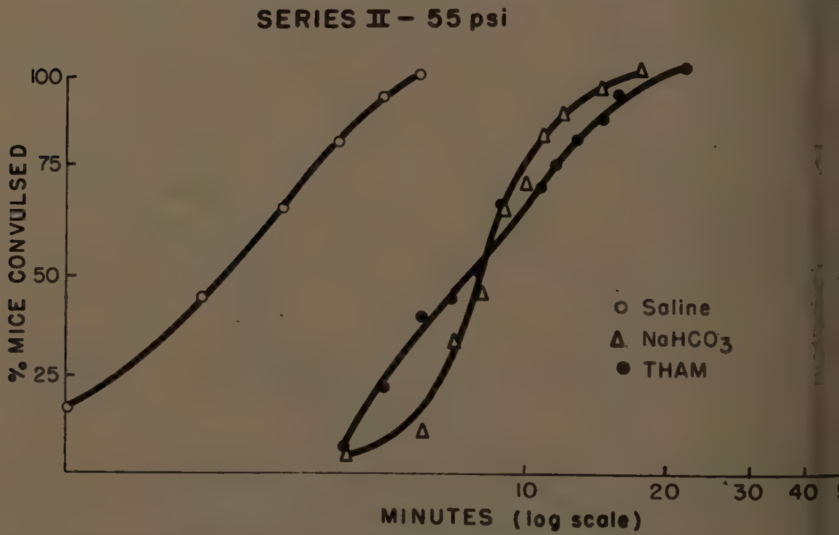


FIGURE 9. Cumulative convulsion times, Series II; 55 psi.

TABLE 8
TWENTY-FOUR-HOUR SURVIVAL OF MICE SUBMITTED TO HIGH O$_2$ PRESSURE

	No. of animals in each group	30 psi (45 min.)	42 psi (45 min.)	55 psi (16 min.)
Saline (control)	18	10	0	2
THAM-treated	18	17	9	17
NaHCO$_3$-treated	18	18	11	15

Discussion

Series I at all oxygen pressures, the injection of THAM caused an increase econvulsion time which was clearly marked. The results with buffered M are more difficult to interpret, and both with this substance and with CO$_3$ there may have been insufficient time allowed for complete absorption istribution. This contention is supported by the much more consistent s obtained in the experiments of Series II, in which three injections were over a period of six hours before exposure, and in which the effects of CO$_3$ were equal to those of THAM. The possible discrepancy introduced the first series by varying compression rates was also avoided in the d series.

e mechanism by which the onset of convulsions is delayed may be com- A considerable number of substances have been tested by various ers to ascertain their effect on the onset of oxygen convulsions and they oo many to list in detail here. As early as 1870, Paul Bert[3] noticed the cular effect of chloroform against convulsions, and drugs that depress the al nervous system seem to have a fairly consistent effect. Thus, for ex- e, H. P. Marks[1] in 1944 reported considerable protection with sodium ide, and less by some of the barbiturates. Pfeiffer and Gersh[4] in the year, of 45 drugs tested, showed that Pentobarbital and Amytal were g the few effective substances. The barbiturates were used by Churchill- dson *et al.*[5] in man to prevent convulsions during prolonged exposure to and four atmospheres of 100 per cent oxygen. Paton and other workers shown that the administration of chlorpromazine, which exerts an anti- athomimetic activity, will protect, and this agrees with the observations rshman *et al.*[6] that removal of the adrenals has the same protective effect. ld,[7] in man, has shown that heat and exercise will speed the onset of symp- while cold and rest will postpone them. Campbell[8] demonstrated a ctive action from thyroidectomy. The administration of insulin, which s the blood sugar, was found by Marks[1] to accelerate symptoms.

e role of carbon dioxide in oxygen toxicity has long been a matter of oversy. It seems likely that there is some retention of CO_2 in the body g exposure to high pressure oxygen. Two factors may be responsible for The first is a decrease of the volume of CO_2 exhaled in the face of rising netric pressure, as pointed out by Bean.[9] The second is the fact that at oxygen tensions sufficient oxygen may be carried in simple solution in the a to meet the body needs without reduction of hemoglobin, impairing O_2 transport by carbamate. In addition, the inhalation of carbon dioxide g exposure to high O_2 tension, speeds the onset of convulsions. These vations seemed to point to carbon dioxide as a causative factor in their rance. This view appeared to be supported by the demonstration by r[10] of greatly raised CO_2 levels as determined by measurements on sub- eous gas depots, and by the findings of Whitehorn and Bean[11] of electro- graphic changes similar to those which would be expected from the nce of excess CO_2.

er evidence, however, does not support this explanation. In 1934 Behnke is co-workers[12] showed in dogs at 45 psi that there was no change in the ombining power of the blood, a change of only a few millimeters in the venous pCO_2, no change in the arterial, and only a very slight drop in venous pH.

Kough *et al.*[13] in 1951 found only a slight rise—to about 50 mm. Hg in depot pCO_2 in man—and a rise of only 3 mm. Hg in internal jugular p accompanied by a fall of 5 mm. Hg in arterial levels. As a result of furt work by Lambertsen and his co-workers,[14] it was concluded that although ficient CO_2 accumulation occurs to account for the hyperpnea seen at h oxygen tensions, convulsions were actually caused by a direct action of oxy on the brain cells. The marked cerebral vasoconstriction that accompa exposure was explained as a protective mechanism that could be parti removed by increases in the inhaled CO_2 that accelerate the onset of sympto Taylor[15] in rats showed no large increase in pCO_2 and a slight fall in *p*H of blood consistent with a metabolic acidosis; he also found evidence of la acid accumulation, and a fall in temperature associated with exposure high O_2 tensions.

The present experiments indicate that by raising the buffering capacity the body fluids, the appearance of some of the signs of oxygen toxicity may delayed. In the first series, a single injection of THAM, 30 min. before c pression, offered some protection, while bicarbonate was much less effect indicating that the diffusion of bicarbonate out of the peritoneal cavity slower than THAM. Such results are in agreement with other observati which show that $NaHCO_3$ distributes slowly through the different body c partments,[16] while THAM distributes widely and much more rapidly.[17] the second series of experiments, where THAM and $NaHCO_3$ were given o a 6-hour period, both agents afforded the same protection. These observati indicate that $NaHCO_3$ will diffuse out of the peritoneal cavity into the vasc spaces within that period of time. The fact that, in this instance, THAM not more effective than $NaHCO_3$ may be due to the fact that the dosage THAM injected had a hypoglycemic effect that limited the protective act of this compound. Indeed, it is known that insulin-induced hypoglycem speeds the appearance of convulsions at high-oxygen tensions. The lower of O_2 uptake observed on mice injected with THAM and bicarbonate (73 cent and 81 per cent of control respectively) should also be considered a possible cause of their protective action against O_2 toxicity. However seems that the results of the first series of experiments could not be enti accounted for by this lowering of metabolic activity. There is also the po bility that THAM may produce an antagonism of sympatho-adrenal funct as shown by Mittelman *et al.*,[18] but this could not account for the protect effect of $NaHCO_3$ in the second series of experiments.

The figures of the survival of the animals after treatment with buffers exposure to high O_2 tension are particularly striking. They indicate that th compounds prevent the occurrence of irreversible damage which occurs in untreated animal.

The mechanisms of protection from high-oxygen tension by administra of buffers are still to be determined. However, as the most important prop of buffers *in vivo* is to correct disturbances of acid base balance, it seems these compounds may exert most of their protective action in oxygen toxi by such a property.

ese experiments, however, do not allow us to assess the respective roles d by CO_2 tension, *p*H, or accumulation of acid metabolites in the genesis acid-base balance disturbances which may develop in extra- and intra- ar compartments as a result of exposure to high-oxygen tension.

Conclusion

ministration of a single injection of 1 ml. of 0.3 *M* THAM intraperi- lly in mice exposed 30 min. later to 30, 42, and 55 psi of oxygen, delays icantly the appearance of convulsions. Isosmolar administration of CO_3 is much less effective. Administration of 3 injections of 1 ml. of 0.3 HAM or 0.3 *M* $NaHCO_3$ over a 6-hour period have an equal effect in ing the appearance of convulsions. These experiments may indicate that xicity is associated with acid-base balance disturbances which can be al- ed to some extent by administration of buffers. The exact nature of the ase balance disturbance of high-oxygen toxicity is still to be determined, ll as the mechanism by which it is corrected by buffers.

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ACUTE AND SUBCHRONIC TOXICITY OF 2-AMINO-2-HYDROXYMETHYL-1,3-PROPANEDIOL

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The investigation of the toxicity of a new drug is a prerequisite to its clin trial. The study provides the information for setting an upper limit to amount of the drug that can be safely administered. Large doses of the d exaggerate the toxic symptoms, thus providing guideposts for the clin investigator. In the present investigation, the toxicity of 2-amino-2-hydro methyl-1,3-propanediol (Tris) was studied.

EXPERIMENTAL

Acute Toxicity

Two variables were of interest in respect to the acute toxicity of Tris. T were (1) the relation between toxicity and *p*H, and (2) the effect of vari additive compounds on the toxicity. The *p*H of a 0.3 *M* Tris solution is 1 and thus some toxicity could be due primarily to alkalinity. To evaluate effect of *p*H, the acute toxicities of a 0.3 *M* Tris solution adjusted to *p*H and to *p*H 5.5 with hydrochloric acid were therefore studied. In addition, effect of adding dextrose and sodium chloride to the Tris solution was inclu in the present study, since hypoglycemia and diuresis have been report following the infusion of large doses of Tris.

Method. Mice were injected intravenously with the test solution in a per of 30 sec. An amount of the solution equal to, 5 ml. above and 5 ml. below approximate LD_{50}, was injected in 10 mice for each dose. The mice w observed for 24 hours, and the LD_{50} was calculated from the per cent of the m that died.

Results. The LD_{50}'s of each of the solutions infused are given in TABL The LD_{50} of 0.3 *M* Tris was 16.5 mmole per kg., which is comparable to the L of 11.5 reported by others.[2] Neither neutralization of the Tris nor additio dextrose or sodium chloride decreased the toxicity of the Tris. In fact n tralization of the Tris appeared to increase the toxicity of the solution. mice that were given a lethal dose convulsed immediately before death.

Subchronic Toxicity

Two rates of administration were investigated. They were (1) rapid jection in 30 sec., as in the acute toxicity study, and (2) slow injection o several hours, as would be employed in humans. Both Tris and Tris neutrali with hydrochloric acid were injected.

Method. (1) Mice and rabbits were injected intravenously in 30 sec. a dosage of 50 and 10 ml. of 0.155 *M* Tris per kg. respectively. Injections w repeated each day for 10 days. Three animals were used for each test.

(2) Rabbits were injected intravenously over a 5-hour period at a dosag about 100 ml. of 0.3 *M* Tris per kg. Injections were repeated each day fo days. Two or three rabbits were used for each test.

lts. (1) The mice and rabbits injected rapidly with the 0.155 M Tris utralized Tris solutions survived for 10 days (TABLE 2). There were no ymptoms, and a histological study of the organs was negative.

The two rabbits infused over a 5-hour period with 0.3 M Tris died after a ys of infusion. Two of the three rabbits infused with Tris adjusted to and one of the two rabbits infused with Tris adjusted to pH 5.5 survived gth of the experiment (TABLE 2). These results indicate that under the ions of the experiment the neutralized Tris is less toxic. The toxic oms noted were anorexia, bloody urine, hindleg paralysis, and irregular

TABLE 1
ACUTE TOXICITY

	LD$_{50}$ in mice	
Solution	ml./kg.	mM Tris/kg.
Tris	55	16.5
Tris (Cl) pH 7.4	43	12.9
Tris (Cl) pH 5.5	29	8.7
Tris in 5% dextrose	48	14.4
Tris in 0.9% sodium chloride	49	14.7
Sodium chloride	120	—

TABLE 2
SUBCHRONIC TOXICITY

Solution	Dose each day	No. of days	Animal	Result
Tris	50 ml./kg. in 30 sec.	10	3 Mice	Nontoxic
Tris (Cl) pH 5.5	50 ml./kg. in 30 sec.	10	3 Mice	Nontoxic
Tris	10 ml./kg. in 30 sec.	10	3 Rabbits	Nontoxic
Tris (Cl) pH 5.5	10 ml./kg. in 30 sec.	10	3 Rabbits	Nontoxic
Tris	79 ml./kg. in 5 hr.	3	Rabbit	Died; hemorrhage
Tris	100 ml./kg. in 5 hr.	2	Rabbit	Died; convulsions
Tris (Cl) pH 7.4	125 ml./kg. in 5 hr.	2	Rabbit	Died; no symptoms
Tris (Cl) pH 7.4	95 ml./kg. in 5 hr.	19	Rabbit	Nonlethal; anorexia
Tris (Cl) pH 7.4	110 ml./kg. in 5 hr.	19	Rabbit	Nonlethal; bloody urine
Tris (Cl) pH 5.5	90 ml./kg. in 5 hr.	1	Rabbit	Sacrificed; compound fracture
Tris (Cl) pH 5.5	100 ml./kg. in 5 hr.	20	Rabbit	Nonlethal; anorexia

tion. Gross observations at autopsy were: abnormally red lungs, is at the point of infusion, bleached liver, darkened spleen, bloated ch, and lesions on the heart and kidney. Histological examination of the was negative. The experiment was not repeated at a lower dose since been reported that a dose of 73 ml. of 0.3 M Tris per kg. for 6 hours in s is not lethal for 21 days.[2]

Accumulation of Tris

development of toxicity after several days of infusion indicated the ility that Tris was accumulating in the rabbits. This possibility was gated.

Method. (1) Four rabbits were injected with 0.3 *M* Tris at a dose of 6
90 ml. per kg. per 3.5 to 5 hours. The Tris blood level was measured (I
and Roberts, elsewhere in this monograph) at the end of the infusion. Or
next day the same amount of Tris was infused. Tris levels were meas
before and after the infusion, and then on the next day; the Tris blood level
measured in the morning and afternoon.

(2) The experiment was repeated using a dose of 5 to 25 ml. of 0.3 *M* Tri
kg. per 2 to 3.5 hours.

Results. (1) Only one rabbit survived the first injection (TABLE 3). In
rabbit, the Tris concentration after the second infusion was higher than a
the first infusion, and the concentration of Tris remaining the next mor
was also higher. Thus the results indicate accumulation.

(2) The rabbits receiving the lower dose did not accumulate Tris; howe
24 hours after the last injection the blood still contained Tris.

TABLE 3
INFUSION OF 0.3 *M* TRIS IN RABBITS

Rabbit	Dose/day	Blood Tris mg./ml.				
		F	BS	AS	MT	A
1	94 ml./kg./5 hr.	2.3	0.38	3.4	0.56	0.
2	90 ml./kg./3.3 hr.	2.8	Died	—	—	–
3	87 ml./kg./2.7 hr.	Died	—	—	—	–
4	59 ml./kg./3.3 hr.	1.9	Died	—	—	–
5	10 ml./kg./2 hr.	0.43	0.15	0.24	—	0.
6	25 ml./kg./2 hr.	0.52	0.16	0.29	0.22	0.
7	24 ml./kg./3.5 hr.	0.98	0.16	1.1	0.34	0.
8	20 ml./kg./3.2 hr.	0.32	0.20	0.66	0.19	0.

Key: F, after first infusion; BS, before second infusion; AS, after second infusion;
morning of third day; and AT, afternoon of third day.

Tris and Glucose Concentration in Blood

Hypoglycemia has been reported after large doses of glucose.[1] It was th
fore of interest to see whether there was any relationship between the con
tration of blood Tris and glucose. The effect of the *p*H of the Tris being infu
was also investigated.

Method. Tris at a level of 0.3 *M* or Tris adjusted to *p*H 5.5 with hy
chloric acid was injected at a dose of 80 and 67 ml. per kg. respectively
hours. The Tris and glucose[3] concentrations in the blood were measured
hour for 7 hours.

Results. With 0.3 *M* Tris, the concentration rose to a maximum of 3.2
per ml. at the end of the infusion and then rapidly dropped (FIGURE 1).
glucose concentration dropped during the infusion and continued to drop
after the infusion. There was no direct relationship between the Tris
glucose concentrations in the blood. With the Tris adjusted to *p*H 5.5,
Tris concentration also rose to a maximum at the end of the infusion (FIG
2) an estimated value of 2.4 mg. (one sample was lost). The Tris concen

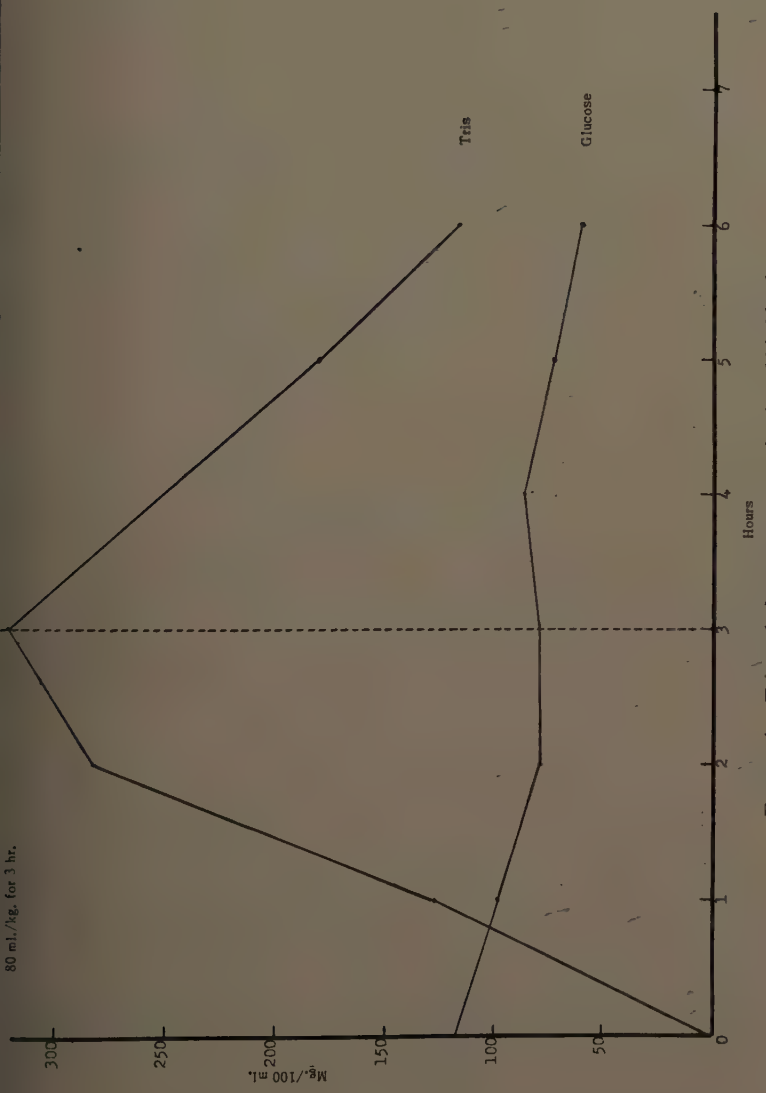
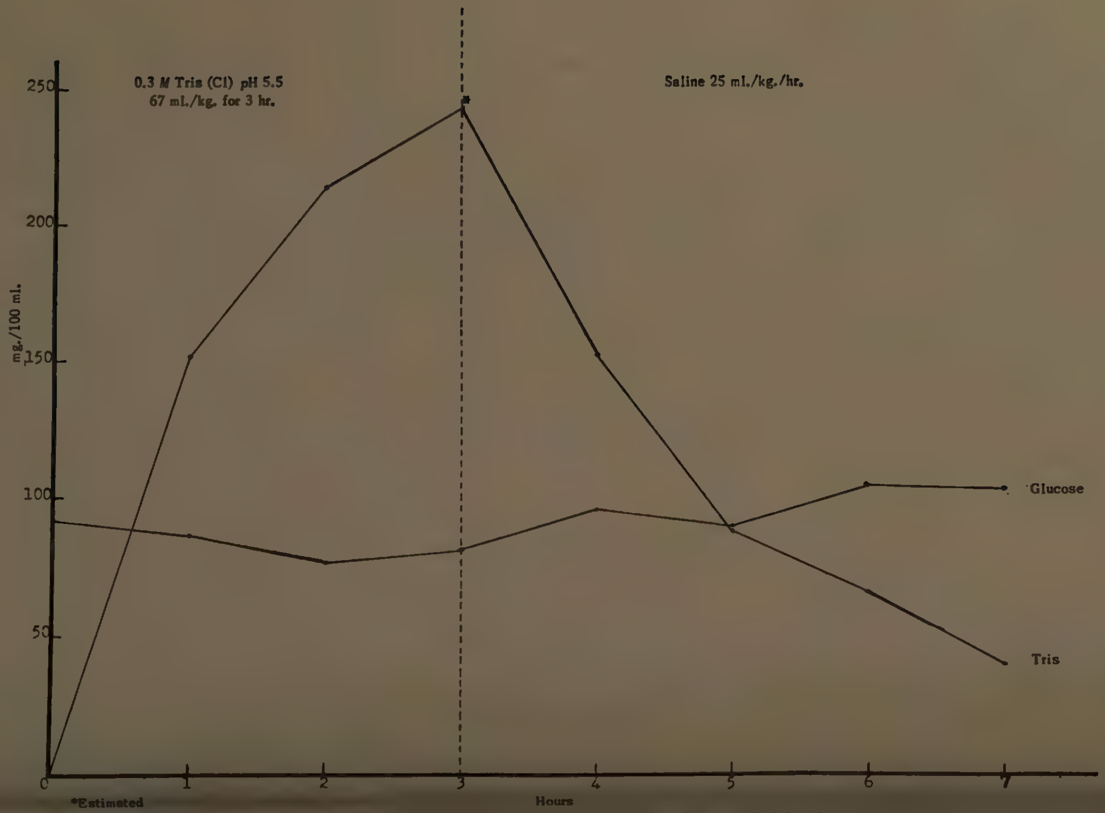


FIGURE 1. Tris and glucose concentration in rabbit blood.


0.3 *M* Tris (Cl) pH 5.5
67 ml./kg. for 3 hr.
Saline 25 ml./kg./hr.
mg./100 ml.
250
200
150
100
50
0
1
2
3
4
5
6
7
Hours
Glucose
Tris

*Estimated

dropped rapidly after the end of the infusion. The glucose concentration ped during the infusion but rapidly returned to normal and then went e normal after the infusion was stopped. Tris and Tris neutralized with ochloric acid both caused hypoglycemia. The hypoglycemic effect of the neutralized with hydrochloric acid was transitory.

Excretion of Tris and Chloride

ethod. In the above experiment on the comparison of Tris and glucose s in the blood, the urine was collected by means of a Foley catheter. Tris chloride[4] content of the urine were measured every hour for 7 hours.

sults. The amount of Tris excreted in the urine reached a maximum at nd of the infusion and dropped rapidly after the infusion (FIGURES 3 and 4). the Tris infusion, only a small quantity of chloride was excreted. With ris adjusted to *p*H 5.5, a larger amount of chloride than Tris was excreted. he end of 7 hours, 44 per cent of the infused Tris was found in the urine with neutralized Tris, 77 per cent was found.

Intradermal Irritation

most of the rabbits infused with 0.3 *M* Tris, necrosis occurred around the of infusion into the marginal ear vein. The cause of irritation was ingated by means of the dye extravasation test.[5]

ethod. An intradermal injection of 0.1 ml. of Tris was made. Trypan was injected intravenously, and the irritation due to the Tris solution was ated by observing the amount of extravasated dye.

sults. The 0.3 *M* Tris was extremely irritating (TABLE 4). Neutralization e Tris with hydrochloric acid reduced the irritation. These results indicate the alkalinity of Tris is probably the cause of irritation.

Effect of Tris on Erythrocytes

utions injected intravenously immediately come in contact with the rocytes. Before injecting a new drug in patients, the effect on the rocytes should be known.

ethod. A drop of rabbit blood was added to 10 ml. of 0.3 *M* Tris. After ır the clear supernatant was examined for hemolysis. This test was red with various concentrations of Tris to determine the concentration at hemolysis first occurred. Solutions of sodium chloride were used as a ol.

sults. There was no hemolysis with 0.3 *M* Tris. Concentrations at which ning and complete hemolysis took place are given in TABLE 5. That conation of Tris which caused beginning hemolysis was nearly twice the conation of sodium chloride (72 mEq. per l.), since Tris is only slightly ionized. djusted to *p*H 5.5 with hydrochloric acid is completely ionized and caused ning hemolysis at a concentration falling between the Tris and sodium de concentrations. Tris caused no immediate deleterious effect on rocytes.

Effect of Tris on Blood Pressure

Method. An anesthetized cat was injected with 1 and then 2 μg. of histami to measure the response of the cat.[6] Five ml. of the test solution were inject and the change in blood pressure was measured. One and then 2 μg. of h tamine were again injected.

Results. The average fall in blood pressure in 3 cats due to injections o and 2 μg. of histamine was 34 and 49 mm. Hg respectively. Tris and T

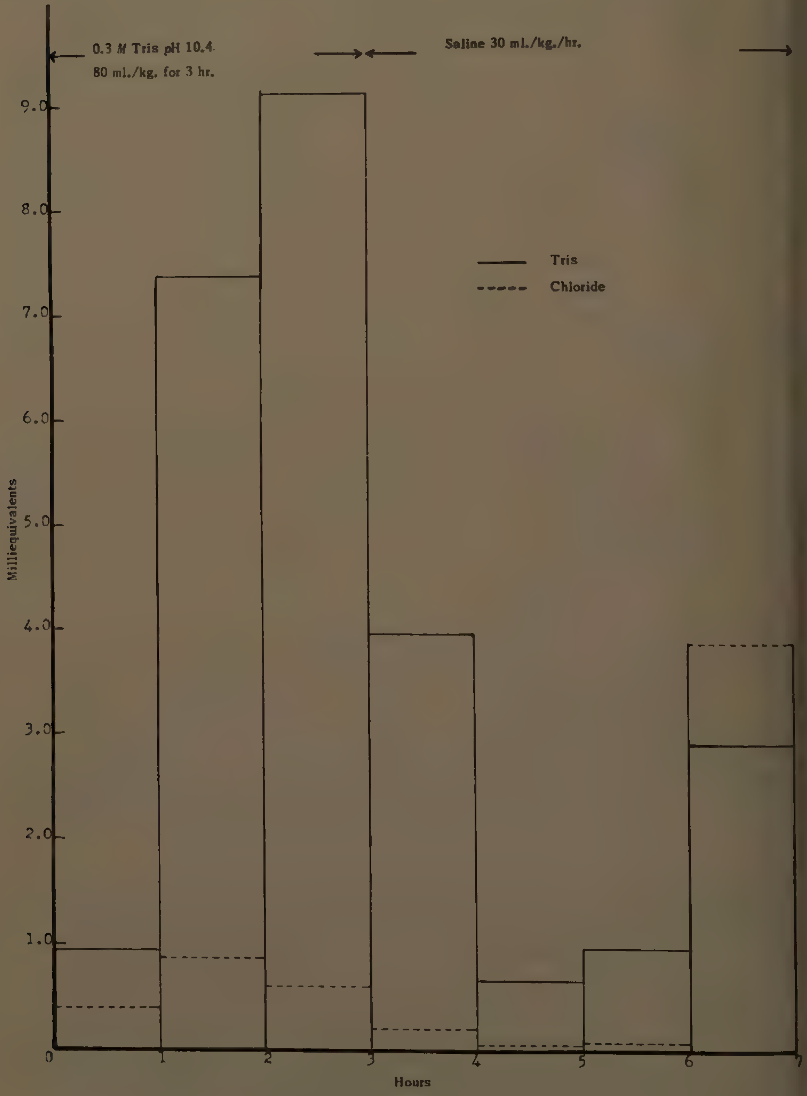


FIGURE 3. Amount of Tris and chloride excreted each hour.

ralized to *p*H 7.4 and 5.5 caused either a slight or no drop in blood pressure. equent injection of 1 and 2 μg. of histamine caused a drop in blood pressure and 54 mm. Hg respectively.

DISCUSSION

e results of this study indicate that the toxic dose of 0.3 *M* Tris lies between d 80 ml. per kg., depending upon the rate administered. It is interesting

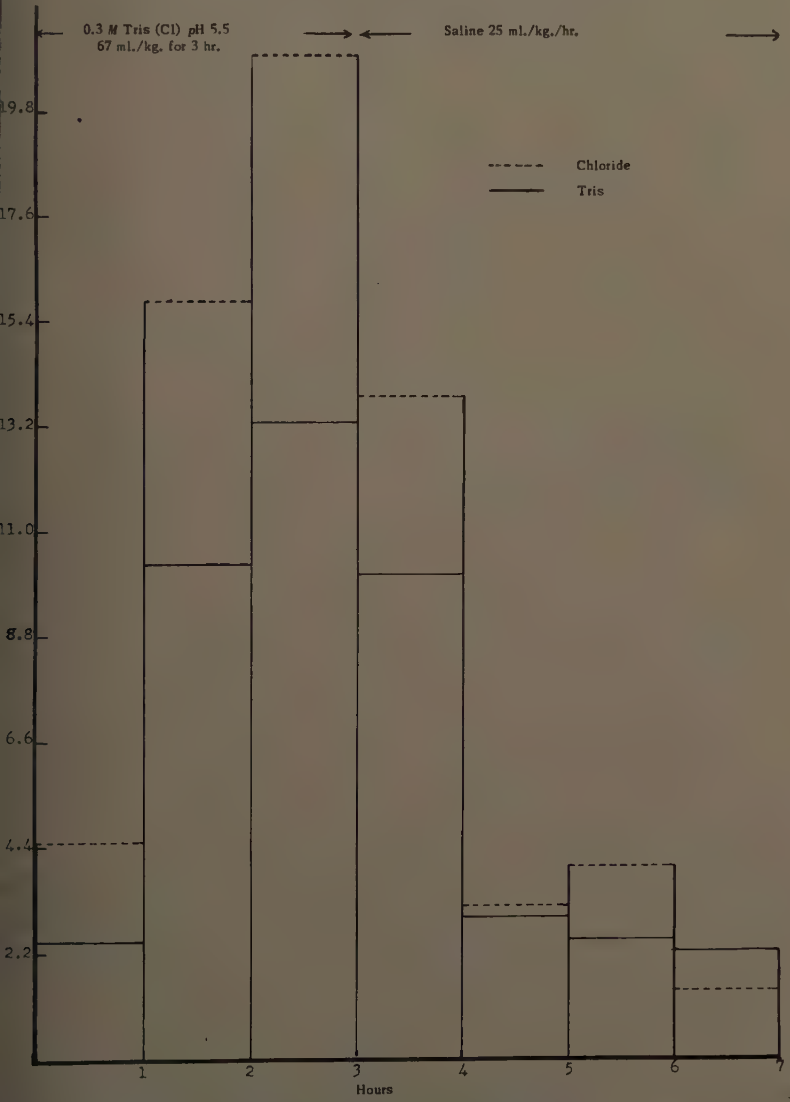


FIGURE 4. Amount of Tris and chloride excreted each hour.

to compare this toxic dose with the doses being used in humans by vario investigators (TABLE 6). The highest dose administered, 30 ml. per kg., about one half the toxic dose. Hypoglycemia accompanied by hunger, profu sweating, and other toxic manifestations were reported following this dose.

Twenty-four hours after an infusion of Tris, some Tris still remained in t animal. This indicates the need for further information concerning t mechanism of Tris excretion. When multiple infusions of Tris at a high dosa are planned, the Tris level in the blood should be followed.

TABLE 4

DYE EXTRAVASATION IN RABBITS

Tris *p*H	Tris concentration			
	0.2 *M*	0.3 *M*	0.6 *M*	1 *M*
10.4	S	S	—	—
7.4	N	N	S	S
5.5	N	N	M	S

Key: S, severely irritating; M, moderately irritating; and N, not irritating.

TABLE 5

HEMOLYSIS OF RABBIT ERYTHROCYTES

Solutions	Hemolysis	
	Slight	Complete
Sodium chloride mEq./l.	72	65
Tris *p*H 10.4 mEq./l.	120	100
Tris (Cl) *p*H 5.5 mEq./l.	100	80

TABLE 6

DOSE IN HUMANS

Reference	Total dose of 0.3 *M* Tris
O'Connor *et al.*[7]	3 ml. per kg. for 1 hr.
Luchsinger *et al.*[8]	9 ml. per kg. for 1 hr.
Berman *et al.*[9]	14 ml. per kg. for 0.5 hr.
Tarail and Bennett[1]	30 ml. per kg. for 1 hr.

Tris at a concentration of 0.3 *M* caused severe irritation when inject intradermally. Reduction of the concentration to 0.2 *M* did not decrease ritation. Thus, it is imperative to prevent extravasation and resulting irritati during an intravenous infusion by placing the needle well within the vein a checking the position of the needle frequently.

SUMMARY

(1) The acute intravenous toxicity of 0.3 *M* 2-amino-2-hydroxymethyl-1 propanediol (Tris) is 55 ml. per kg. Neither neutralization of Tris with h drochloric acid nor addition of glucose or sodium chloride decreases the toxici

The maximum subchronic nonlethal dose of 0.3 M Tris in rabbits is be-
n 75 and 90 ml. per kg. administered in 5 hours. Neutralization of the
with hydrochloric acid decreases the subchronic toxicity of Tris.

Twenty-four hours after an infusion of Tris, some Tris still remains in
nimal. If high doses of Tris are given frequently, the Tris will accumulate.

The injection of high doses of Tris causes hypoglycemia that persists
the infusion of Tris. Tris neutralized with hydrochloric acid causes
glycemia only during the infusion. There is no direct relationship between
and glucose concentrations in the blood.

The intradermal injection of 0.1 ml. of 0.3 M Tris causes severe irritation.
ralization of the Tris before injection reduces the amount of irritation.

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DISCUSSION

P. MERRILL (*Harvard University, Cambridge, Mass.*): It should be recalled
that hydropic degeneration of the renal tubules has also been observed
n hypertonic solutions of saline or glucose were administered to patients
ring from diabetic acidosis. In the present instance the concentration of
AM administered was 1.5 molar or 5 times the osmolality of body fluids.

ABRIEL G. NAHAS: The high concentration of the solution of THAM ad-
stered, as well as the rapidity of infusion, may account in part for the
us thrombosis that was observed in the patients reported in this paper.
ous thrombosis has not been a serious problem in our experience when a
M solution of THAM was administered at the rate of 1 to 10 ml./min. in
ge antecubital vein in an adult through a 19-gauge needle or an indwelling
eter threaded in a large vein. Infusions through foot or hand veins have
ys been unsuccessful and have been accompanied by spasms and extrava-
n. Furthermore, there are now micromethods for pH and $p\text{CO}_2$ deter-

mination that can be performed in 0.2 to 0.5 ml. of "arterialized" blood
tained from peripheral veins of the heated hand or even from puncture of t
earlobe. Though technical difficulties of administration may represent
serious difficulty in the therapy with THAM, it appears that its routine clini
use should await further investigation concerning its fate in the body and ra
of excretion.

VI. Clinical Use of Amine Buffers

E USE OF 2-AMINO-2-HYDROXYMETHYL-1,3-PROPANEDIOL IN THE CORRECTION OF METABOLIC AND RESPIRATORY ACIDOSIS

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has already been demonstrated at this conference that 2-amino-2-hydroxy- yl-1,3-propanediol (THAM) can effectively control acidosis in animals man. Therefore, we shall not dwell on our own preliminary experimental , which showed that respiratory acidosis could be temporarily controlled e use of THAM whether given by gastric tube or intravenously. How- we propose to demonstrate one aspect: namely, the depression of ventila- that occurs during THAM infusion.

ree patients with respiratory acidosis were given 20 gm. of THAM in- nously over a 40-min. period (FIGURE 1). During the infusion of THAM, rterial *p*H rose in all cases, while the minute ventilation fell. While it is shown in FIGURE 1, the arterial oxygen tension (PaO_2) fell 11 to 25 mm. ury during the THAM infusion. The decrease in PaO_2 was greater than l be accounted for by the shift in the hemoglobin dissociation curve, which rs secondary to the change in *p*H, but was due mainly to the decreased ilation. As many patients with respiratory acidosis also have arterial en unsaturation, treatment with THAM may be potentially dangerous ss care is taken to offset the effect of this respiratory depression by simul- usly giving oxygen. That this can be accomplished is shown in the fol- g patient.

is man (FIGURE 2) had severe diffuse obstructive emphysema. At rest as breathing about 10 l./min. He was then given 100 per cent oxygen, h by removing the anoxic stimulus to respiration, resulted in his minute ilation falling to about 7 l./min. Arterial blood specimens were taken e the start of oxygen and 20 min. after. The patient was then allowed to for about 1 hour. At this time his ventilation was about 8 l./min. He again given oxygen, but this time was simultaneously given THAM. His rations were now depressed to under 6 l./min. Again, arterial bloods were n at the start and toward the end of the oxygen administration.

terial blood studies on this patient are reported in FIGURE 3. The first nn represents the findings at rest and shows a mild degree of acidosis. e second column are studies done after 20 min. of O_2 administration. The obvious change is a decrease in minute ventilation of about 2.5 l. After min. of 100 per cent oxygen and THAM, the patient became alkalotic; rterial carbon dioxide tension ($PaCO_2$) fell; but he maintained 100 per oxygen saturation despite a decrease in ventilation to 6 l./min. After 20 of breathing THAM and 100 per cent oxygen, he was markedly alkalotic a *p*H of 7.56; the $PaCO_2$ was further reduced. Ventilation was reduced 7 l./min., and yet was maintained 100 per cent oxygen saturation in the ial blood.

It is apparent that as long as the patient is given oxygen, the anoxic eff of ventilatory depression can be offset. The question now arises as to wheth the ventilatory depression which occurs with THAM is due to the lowering the carbon dioxide tension, removing one of the major stimuli to the respi

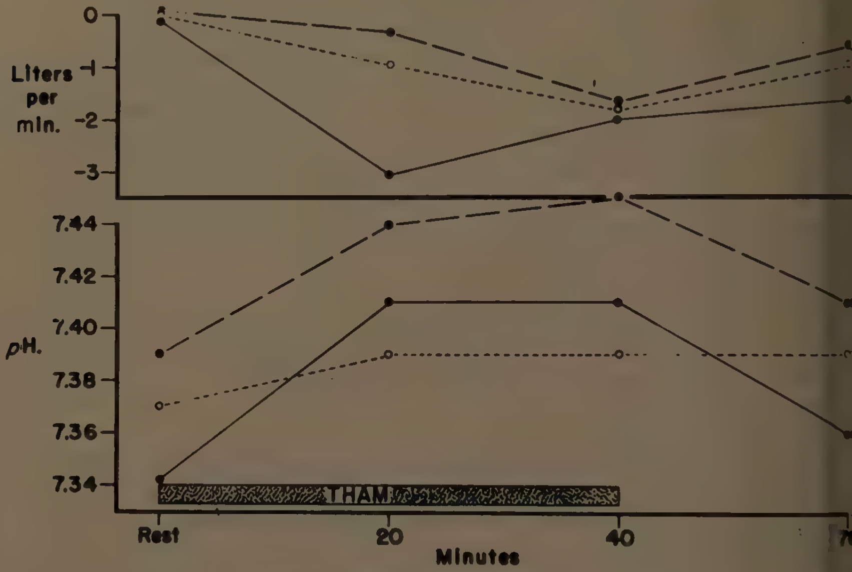


FIGURE 1. See text.

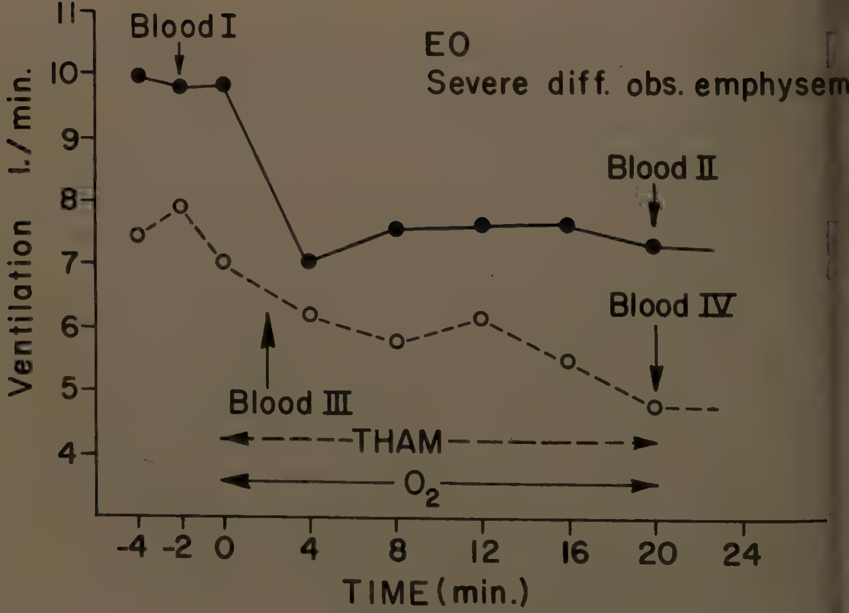


FIGURE 2. See text.

center, or whether it is due to the associated rise in *p*H. An attempt to er this question was made in the next experiment.

is was a young adult male recovering from minimal pulmonary tubercu- He was given 5 per cent carbon dioxide oxygen to breathe, and his venti-

.	I.	II.	III.	IV.	V.
	Rest	100% O_2 20 min.	100% O_2 & THAM 2 min.	20 min.	O_2 & THAM stopped 20 min. later
	7.35	7.34	7.45	7.56	7.44
2	43.5	46.5	39	35.5	45
2	20.70	21.50	22.40	25.50	25.50
	95	100 +	100	100 +	86
	9.88	7.40	6.0	4.70	

GURE 3. See text. Reproduced by permission of *The American Journal of the Medical ces.*[2]

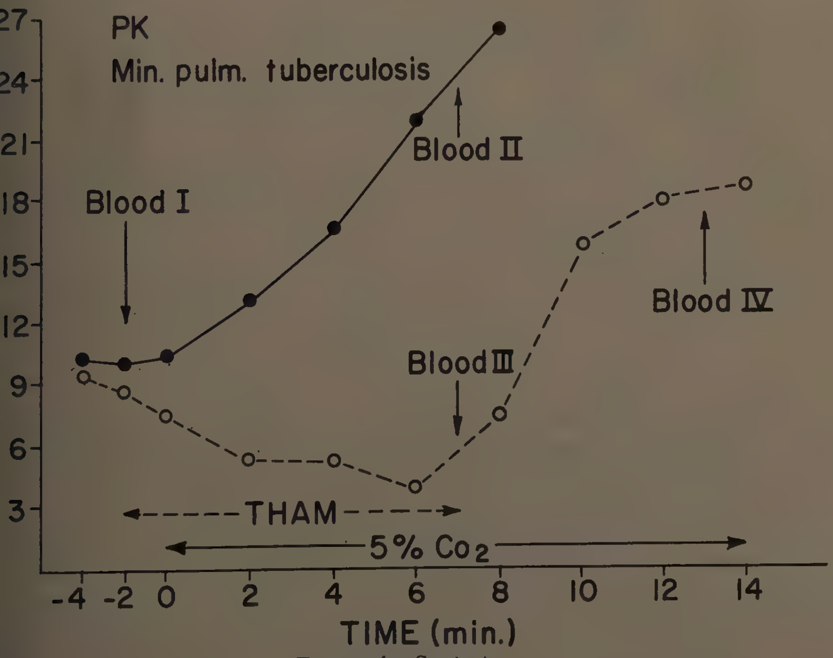


FIGURE 4. See text.

on increased from 10 l./min. to 27 l./min. within an 8-min. period (FIGURE After his respiration returned to normal, he was again given 5 per cent oon dioxide and oxygen to breathe, but at the same time he was given nfusion of 36 gm. of THAM over an 8-min. period. Instead of the anticipated increase in ventilation, his minute ventilation was actually decreas The THAM was stopped, but the carbon dioxide inhalation continued, a his ventilation immediately increased. Arterial blood specimens were tained at the points marked, and the results are shown in FIGURE 5. At r arterial blood findings were normal. After breathing 5 per cent carbon oxide and oxygen for 7 min., the patient had respiratory acidosis with a mark increase in ventilation. He was then given THAM along with the inhalat of carbon dioxide for 7 min. He maintained a normal *p*H, as his $PaCO_2$ r to 50 mm. of mercury. His carbon dioxide content increased, but ventilat was markedly decreased. So despite a rise in $PaCO_2$, ventilation did not crease.

It would appear that in order to prevent increased alkalosis resulting fr hyperventilation with consequent increased excretion of carbon dioxide,

P.K.	I.	II.	III.	IV.
	REST	5% Co_2 7 min.	Co_2 & THAM 7 min.	THA stopp 6 mi
*p*H	7.40	7.35	7.42	7.
Pco_2	40	45	50	48
Cco_2	21.20	21.50	27.30	26.
So_2	96.2	100 +	100 +	100
V	10.15	23.4	5.3	17.

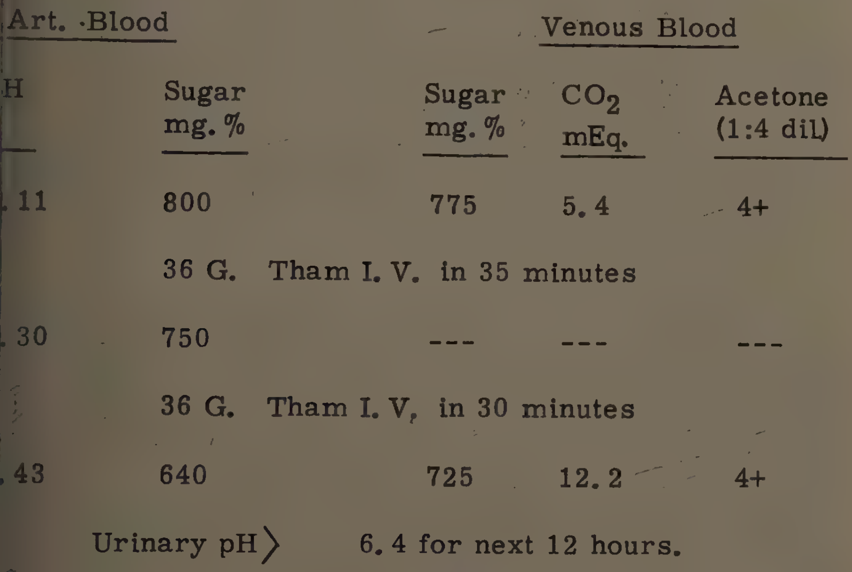
FIGURE 5. See text.

*p*H becomes the dominant controlling influence and actually depresses venti tion. It has been our experience during THAM infusion that whenever *p*H rose above normal, ventilation was depressed. In the early stages of us this drug, and before we knew its full therapeutic effect, we produced respi tory arrest on one occasion, fortunately without ill effect.

Therapeutically, THAM has proven useful for short-term control of respi tory acidosis, but due to problems of administration, to be mentioned later has not been possible to use it for more than a few hours at any one time.

Now turning our attention to metabolic acidosis, our experience has b limited. We have used THAM in one case of uremic acidosis in which it fectively corrected the *p*H for several hours but, in so far as we could t there was no beneficial clinical effect.

In view of the reported hypoglycemic effect of THAM and its ability to a correct acidosis, THAM might prove a very useful drug in the treatment betic ketoacidosis. We have used it in 3 such patients. The first patient s a man aged 47, who had had diabetes for 5 years. He also had rheumatic vular heart disease and congestive heart failure. He was admitted in deep na with a venous blood sugar of 775 mg., marked acetonemia and an arterial of 7.11. The blood urea nitrogen 3 weeks prior to this admission was 42 . He received no insulin, but was given 36 gm. of THAM in 200 cc. of ter (FIGURE 6) over a 35-min. period. At the end of this time, his *p*H had en to 7.30; his arterial blood sugar had fallen to 750 mg. He was then given other 36 gm. over the next half hour, receiving altogether 72 gm. of THAM 1 hour. At the end of this time, his *p*H was 7.43; the arterial blood sugar s 640 mg.; although the venous blood sugar had fallen only from 775 mg. 725 mg. His urinary *p*H was 6.4, and remained above this level for the xt 12 hours. Following the THAM infusion he was treated in a routine anner, receiving 800 units of insulin over the next 24 hours. He appeared to doing quite well, but after 18 hours he developed renal shutdown and died hours later. At autopsy he showed rather unusual findings, which will be scribed in case others may have had the same experience.



Art. Blood		Venous Blood		
H	Sugar mg. %	Sugar mg. %	CO_2 mEq.	Acetone (1:4 dil)
.11	800	775	5.4	4+
	36 G. Tham I. V. in 35 minutes			
.30	750	---	---	---
	36 G. Tham I. V, in 30 minutes			
.43	640	725	12.2	4+

Urinary pH 〉 6.4 for next 12 hours.

FIGURE 6. See text.

In the kidneys, the lining of the proximal tubules showed marked swelling d hydropic degeneration, with almost complete obstruction of the lumen the tubules as a result. Similar changes occur in the Ammanni-Epstein ndrome, which is a rare autopsy finding and usually occurs in patients dying diabetic coma who have not received insulin. However, the changes in the nmanni-Epstein syndrome are mainly in the distal part of the tubule, and t in the proximal part as occurred in this patient.[1] This patient also showed rked abnormality in the liver, with hydropic degeneration of the cells,

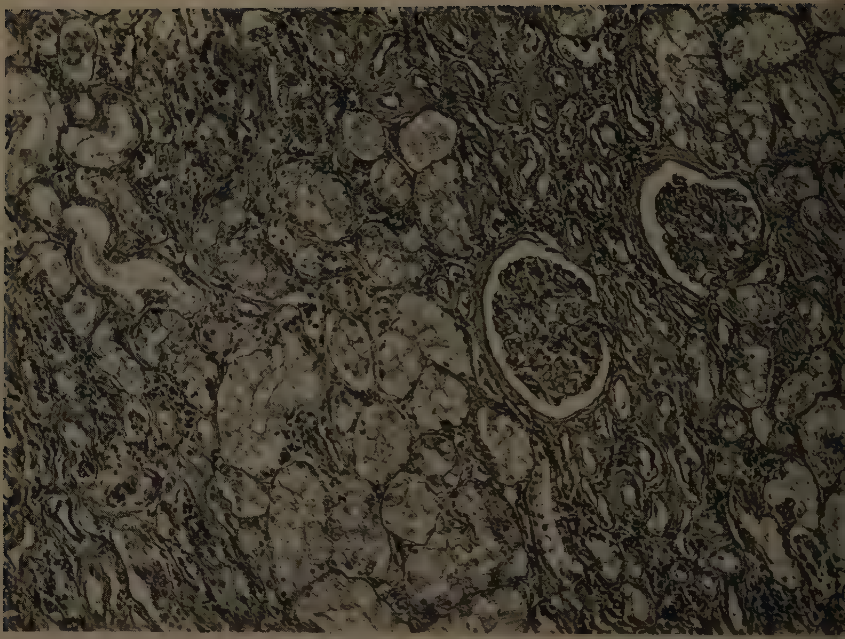


FIGURE 7. Photomicrograph of the kidney in Case 1, showing change in tubules. ×15

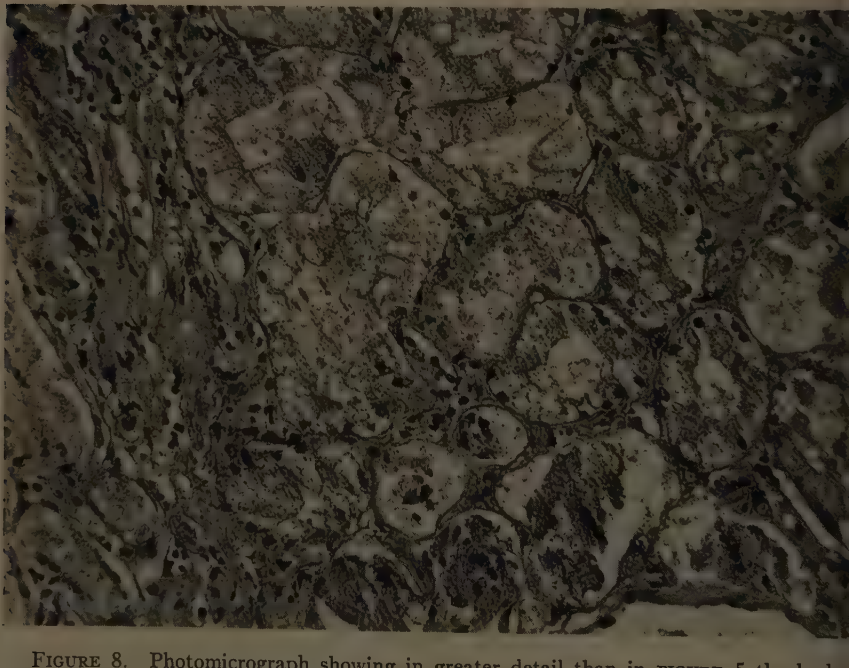


FIGURE 8. Photomicrograph showing in greater detail than in FIGURE 5 the hydro degeneration of tubular cells. ×325.

ough the normal architecture was not destroyed. These liver changes have been reported in the Ammanni-Epstein syndrome (FIGURES 7, 8, and 9).

he second patient in diabetic coma was admitted in a moribund state. One k prior to this final illness, the blood urea nitrogen was normal. The blood ar was 1400 mg. She was given 200 U. of insulin, and then, one-half hour r, 72 gm. of THAM over a 90-min. period, at which point she became scious. She was then treated in a routine manner with insulin and intraous fluids, but died 12 hours after completion of the THAM administration. autopsy her kidneys showed the identical changes we have seen in the vious case, but the liver was normal.

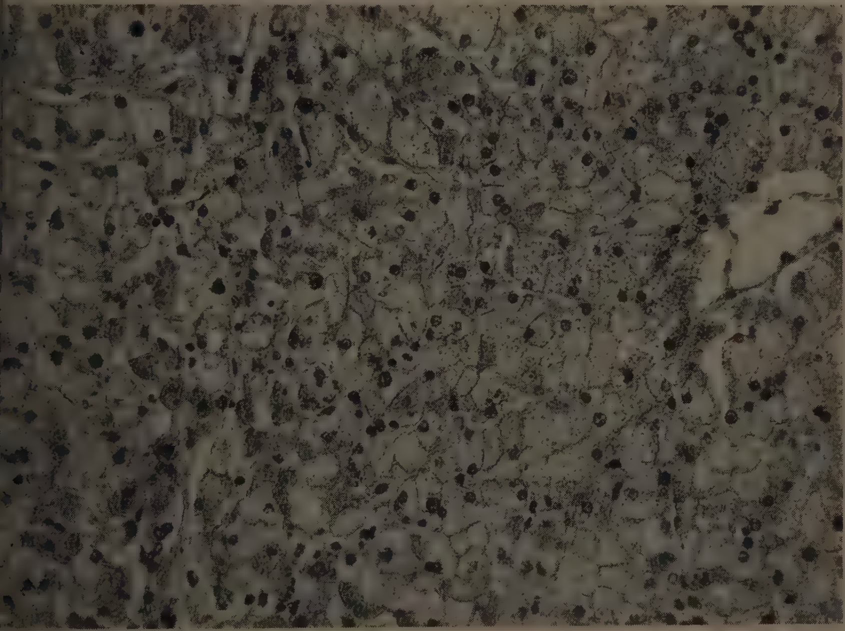


FIGURE 9. Hydropic degeneration in liver cells. ×325.

While the renal changes may have been a variant of the Ammanni-Epstein ndrome, it would be rather a strange coincidence that such a rare pathological ding should occur in two consecutive patients. In any case, this would not count for the changes in the liver seen in the first patient. Whether, however, there is any relationship between the THAM administration and these dings is hard to say. We have treated one other patient with ketoacidosis whom the acidosis was corrected without any apparent effect on the patient.

It has been shown that THAM can correct acidosis either due to respiratory metabolic causes. Before THAM can be used as a routine method of rapy, however, a more satisfactory means of controlling the dosage must found, and also a more satisfactory method of administration. At present is necessary to give it either by gastric tube, in which case diarrhea always develops within a few hours; or, if given intravenously, thrombosis or ext vasation with resulting sloughing of the subcutaneous tissues occurred. control the rate of administration, furthermore, it is necessary to measure t arterial pH, using an indwelling arterial needle, and most patients object this after a few hours. Therefore, until these two problems of administrati and dosage control can be solved, this buffer will probably not attain any w clinical use.

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HE USE OF 2-AMINO-2 HYDROXYMETHYL-1,3-PROPANEDIOL IN THE MANAGEMENT OF RESPIRATORY ACIDOSIS*

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d the Department of Medicine, Georgetown University Medical School, Washington, D.C.

he respiratory effects of 2-amino-2-hydroxymethyl-1,3-propanediol HAM) in essentially normal individuals were reported earlier by us.[1] In ordance with the results obtained by Nahas *et al.*[2] in dog experiments, the avenous infusion of this particular amine buffer resulted in an increase in sma bicarbonate and *p*H. Concomitantly with these changes a fall in ute ventilation occurred. The reduction in minute ventilation affected pre- ninantly the alveolar ventilation, as indicated by a rising arterial carbon xide tension. Alveolar hypoventilation during THAM infusion was ac- npanied by a fall in respiratory quotient. This resulted in an alveolar gen tension that was lowered more than the alveolar or arterial carbon xide tension. The low alveolar oxygen tension was reflected in anoxemia. en though these findings did not suggest the use of THAM in the treatment patients with respiratory acidosis, a series of 12 patients with chronic ob- ictive emphysema was studied in order to delineate more clearly the use- ness of this amine buffer in patients with chronic alveolar hypoventilation, l to evaluate the respiratory regulatory mechanisms occurring in this type pulmonary insufficiency during the infusion of THAM.

Materials and Methods

ll 12 of the patients studied had chronic obstructive emphysema and onic alveolar hypoventilation. They were divided into two groups: Group TABLE 1) consisted of 7 patients with uncompensated respiratory acidosis, indicated by a *p*H of 7.35 or below and an arterial carbon dioxide tension 50 mm. Hg or above; Group II (TABLE 2) consisted of 5 patients with com- nsated respiratory acidosis, that is, with a *p*H value of the arterial blood of 9 or above and a carbon dioxide tension of 45 mm. Hg or above. All sub- ts were studied at bedside after overnight fasting. They were without medication. Since the clinical condition of the patients did not permit ailed ventilatory tests, only arterial blood studies were carried out. These dies consisted of the determination of oxygen tension using the Clark ctrode as adapted by Severinghaus, ascertainment of *p*H measurements ng a capillary glass electrode and a Metrohm *p*H compensator, and determin- on of the carbon dioxide tension using the Severinghaus P_{CO_2} electrode with Instrumentation Laboratories P_{CO_2} meter. Arterial blood samples were wn during a control phase at 15 min. intervals; an intravenous infusion s then started that contained THAM in 0.3 *M* concentration as well as nM/l. KCl and 30 mM/l. NaCl. The infusion was adjusted to an approxi- te rate of 100 mM/hour. Arterial blood samples were drawn at intervals

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TABLE 1

Group I Patient		Control (min.)		THAM Infusion* Peroid												After THAM was discontinued (min.)		
				THAM (min. after onset of THAM infusion)					Oxygen by mask (min. after onset of oxygen)				Prethcamid† (min. after onset of dosage)					
		−15	0	15	30	60	90	120	15	30	60	90	15	30	60	30	60	90
G.G.	*p*H	7.35	7.35		7.36	7.37		7.43		7.44	7.45			7.45‡	7.43‡	7.40	7.44	7.45
Age: 63	$PaCO_2$	45.8	44.3		46.0	47.3		45.0		46.7	45.2			47.2‡	52.5‡	55.7	48.5	49.0
N/M	PaO_2	63	61		60	50		50		404	398			404‡	377‡	387‡	52	52
C.M.	*p*H	7.32	7.33	7.33	7.34	7.36	7.40	7.38		7.26	7.28	7.29		7.39‡	7.36‡	7.40		
Age: 63	$PaCO_2$	58.5	62.5	61.3	59.8	57.8	58.0	60.8		75.0	74.5	76.3		63.6‡	67.0‡	59.3		
W/M	PaO_2	57	57	52	52	51	50	47		415	338	297		162‡	180‡	48		
B.H.	*p*H	7.35	7.35	7.35	7.38	7.38	7.38	7.41	7.32	7.32	7.28	7.26					7.34	7.35
Age: 60	$PaCO_2$	70.5	63.6	66.0	63.6	65.0	65.2	62.2	82.0	80.5	91.7	93.3					76.0	72.8
W/M	PaO_2	43	44	37	34	34	30	30	233	291	285	294					29	31
A.F.	*p*H	7.34	7.35	7.36	7.37	7.38	7.39	7.39								7.38§	7.34§	
Age: 38	$PaCO_2$	49.5	47.6	45.2	47.5	47.2	47.4	46.3								47.5§	52.7§	
W/M	PaO_2	44	46	46	41	44	45	44								53§	57§	
W.F.M.	*p*H	7.34	7.36	7.39	7.39	7.39	7.41	7.40								7.40	7.40	
Age: 34	$PaCO_2$	60.0	58.0	53.5	54.8	53.4	53.2	50.5								51.0	51.6	
W/M	PaO_2		44	47	46	39	37	34								37	38	
A.W.	*p*H	7.35	7.36	7.39	7.35	7.38	7.41	7.40					7.41	7.41	7.43	7.40		
Age: 57	$PaCO_2$	70.2	70.2	70.0	68.3	67.2	67.0	64.3					64.3	62.4	62.0	62.5		
W/M	PaO_2	54	51	45	46	41	44	44					42	45	38	39		
C.T.C.	*p*H	7.36	7.35		7.41	7.39												
Age: 63	$PaCO_2$	60.2	62.6		55.7	56.3												
N/M	PaO_2	50	46		41	41												

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* Approximately 100 mM/hour.

† Approximately 600 mg./hour.

TABLE 2

GROUP II Patient		Control (min.)		THAM INFUSION* PERIOD												After THAM was discontinued (min.)		
				THAM (min. after onset of THAM infusion)					Oxygen by mask (min. after onset of oxygen)				Prethcamid† (min. after onset of dosage)					
		−15	0	15	30	60	90	120	15	30	60	90	15	30	60	30	60	90
R.L.	*p*H	7.39	7.39	7.43	7.44		7.44	7.45									7.41§	
Age: 52	PaCO$_2$	46.5	44.0	43.7	44.6	47.5	47.3	47.1									54.3§	
W/M	PaO$_2$	49	49	47	45	42	45	45									79§	
H.L.	*p*H	7.42	7.41	7.43	7.44	7.43	7.44	7.46	7.43	7.43	7.42						7.39	
Age: 63	PaCO$_2$	47.5	48.5	46.5	44.3	52.4	50.0	50.8	54.5	58.3	58.5						62.8	
W/M	PaO$_2$	41	40	33	33	38	38	37	215	234	253						278	
H.A.T.	*p*H	7.40	7.40		7.42	7.43		7.43		7.40	7.43			7.45§	7.44‡	7.41§	7.43	7.43
Age: 62	PaCO$_2$	45.0	46.0		49.3	47.0		47.2		50.5	52.8			50.4§	54.0‡	55.3§	51.7	52.4
W/M	PaO$_2$	49	47		37	37		35		300	311			279§	253‡	222§	37	36
A.G.W.	*p*H	7.40	7.40	7.41	7.41		7.43	7.42		7.35	7.37					7.40	7.40	
Age: 65	PaCO$_2$	58.5	59.3	63.3	60.5	61.0	62.0	63.3		81.5	82.0					65.0	66.2	
W/M	PaO$_2$	43	45	37	40	37	33	38		296	352					46	41	
A.D.H.	*p*H	7.40	7.40	7.43	7.45	7.44	7.47	7.48		7.46	7.46						7.41	7.45
Age: 70	PaCO$_2$	46.0	47.2	46.0	48.8	50.8	48.0	50.5		54.3	56.0						57.5	51.0
W/M	PaO$_2$	67	59	55	54	48	55	49		459	407						49	50

* Approximately 100 mM/hour.

† Approximately 600 mg./hour.

‡ Prethcamid plus oxygen.

§ Oxygen, 3 l. per min. by nasal catheter.

of 15, 30, 60, 90, and 120 min. after onset of the infusion. During this per the patients breathed room air. Following the sampling of the 120-m specimen of blood, 7 patients were given pure oxygen by mask while the fusion was continued. Blood samples were then drawn at intervals of 30 60 min. and, in 2 cases, at intervals of 90 min. after the onset of oxygen brea ing. In 4 patients Prethcamid,* a respiratory stimulant, was added to infusion towards the end of the oxygen breathing period. The amount Prethcamid given approximated 600 mg. per hour.

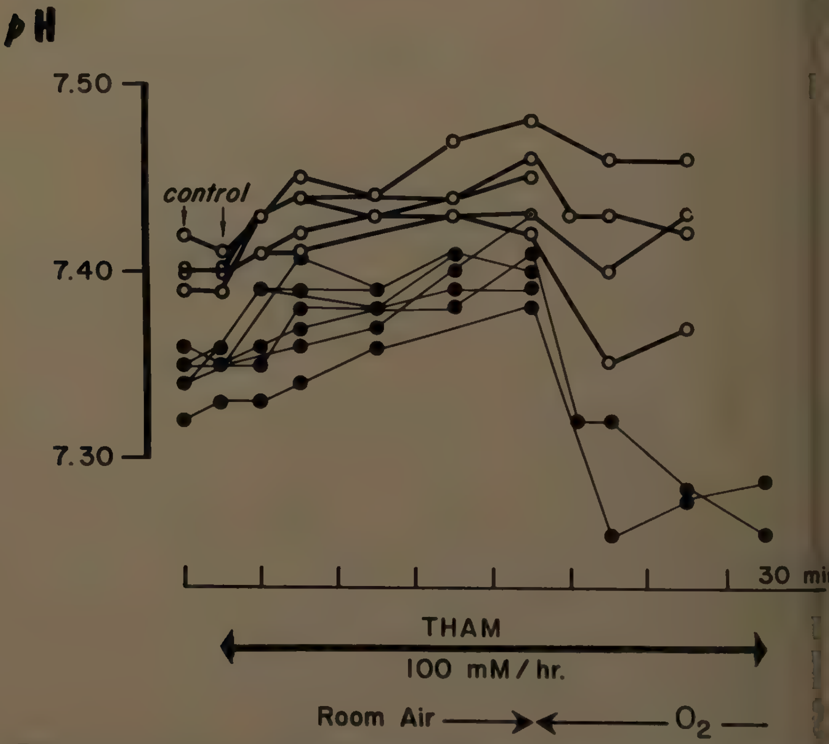

FIGURE 1. Arterial *p*H values of 5 patients with compensated respiratory acidosis (*o circles*) and 7 patients with uncompensated respiratory acidosis (*dots*) during THAM infu while breathing room air and while breathing oxygen.

Results

The results of our study of these 12 patients are tabulated in TABLES and 1B.

THAM: Room Air Breathing

It is noted that the infusion of THAM resulted in a rise of the *p*H in all patients. This reflects a relative metabolic alkalosis, since there was an crease in the bicarbonate content primarily. In Group I (patients with

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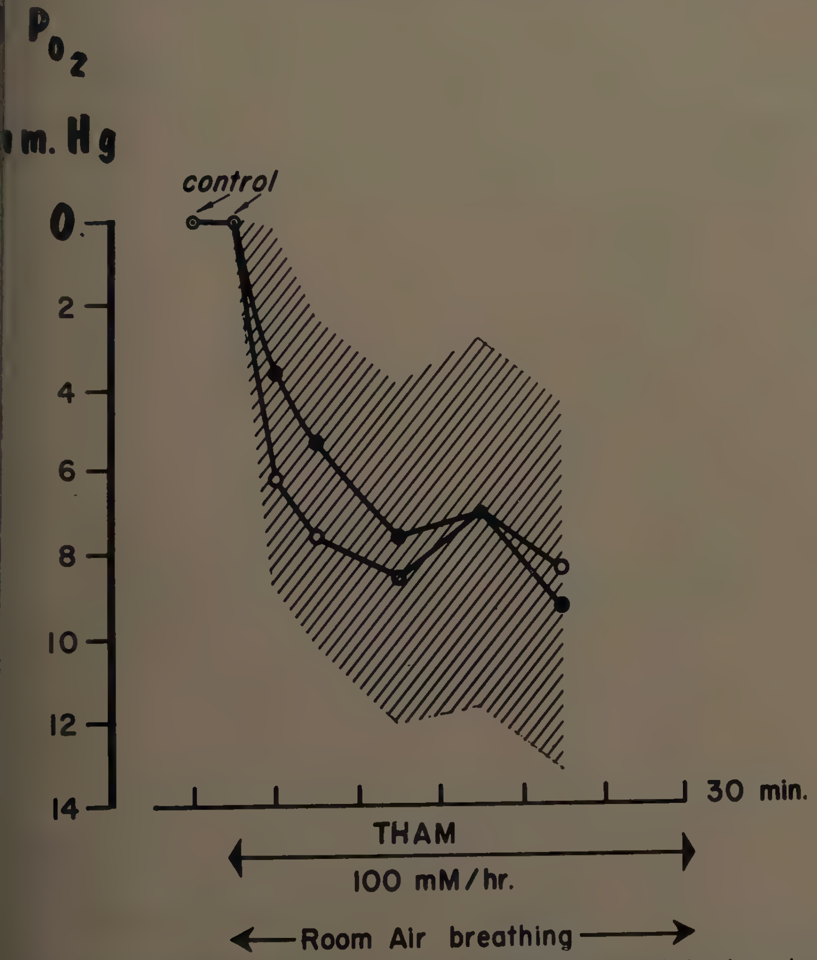
* Kindly supplied by Ethicon, Inc., Somerville, N.J.

ensated respiratory acidosis), the *p*H approximated normal towards the
f the room air breathing period. In Group II (patients with compensated
ratory acidosis), the *p*H rose to alkalotic levels in all instances (FIGURE 1).
e oxygen tension showed a marked fall in all patients. There was no



IGURE 2. Mean changes in arterial Po_2 observed during THAM infusion in patients
compensated respiratory acidosis (*open circles*) and uncompensated respiratory acidosis
) while breathing room air. The shaded area represents plus or minus one standard
tion of the mean of both groups.

ificant difference in the change in oxygen tension in the two groups (FIGURE

he carbon dioxide tension fell slightly in all patients who had uncompen-
d respiratory acidosis. In patients with compensated respiratory acidosis
oup II), on the other hand, a rise in carbon dioxide tension during the period
HAM infusion was observed (FIGURE 3). The difference of behavior in

these two groups is statistically significant in the 60-, 90-, and 120-min samples (FIGURE 4).

THAM: Oxygen Breathing

During the period of oxygen breathing all but one patient showed a rise carbon dioxide tension and a fall in *p*H, indicating an accentuation of the p

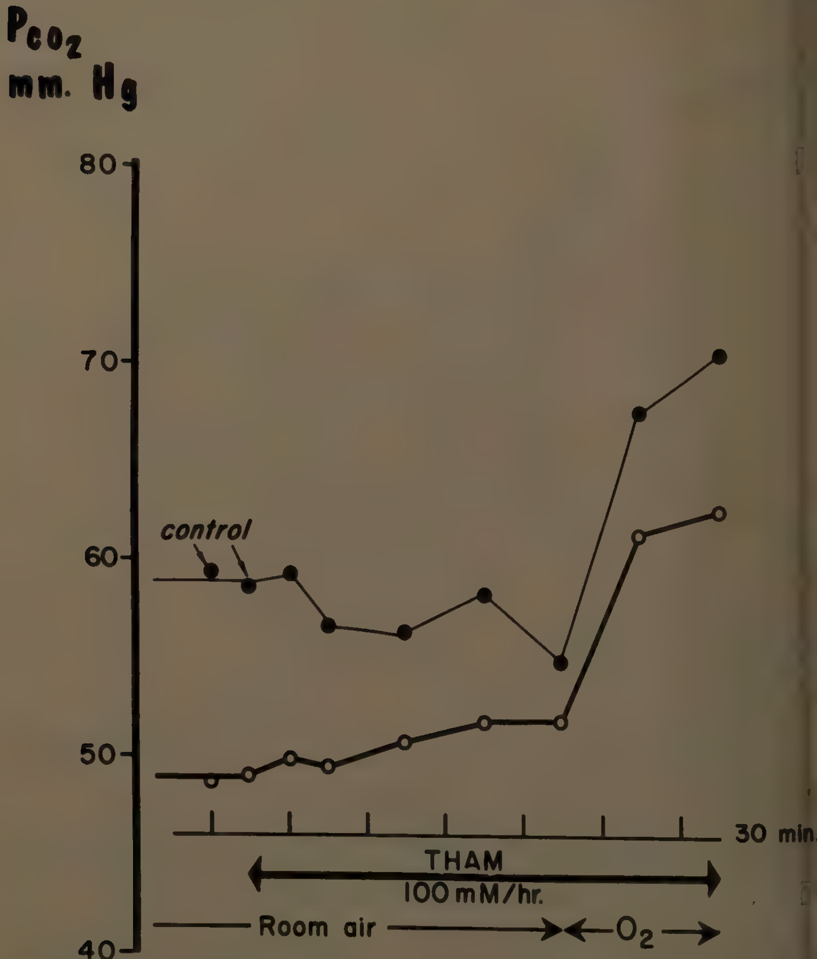


FIGURE 3. Mean arterial P_{CO_2} values of patients with compensated respiratory acid (*open circles*) and uncompensated respiratory acidosis (*dots*) during THAM infusion w breathing room air and while breathing oxygen.

existing respiratory acidosis. The oxygen tension rose in all instances t level above 200 mm. Hg.

THAM + Prethcamid

One of four patients receiving a respiratory stimulant (Prethcamid), gether with the THAM infusion showed a marked increase in alveolar ve

ion, as indicated by the rising *p*H and falling arterial carbon dioxide tension.
e other three patients showed little or no respiratory stimulatory effect as
lged from the arterial blood gas composition.

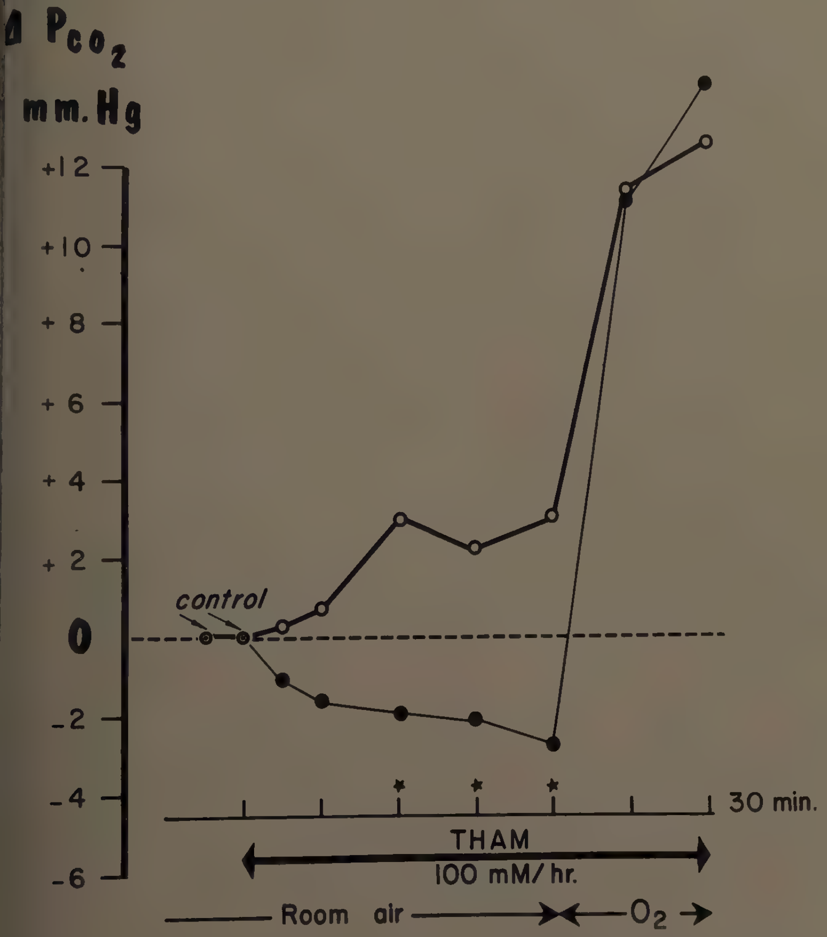


FIGURE 4. Mean changes in arterial P_{CO_2} of the group with compensated respiratory aci-
sis (*open circles*) and uncompensated respiratory acidosis (*dots*) during THAM infusion
ile breathing room air and oxygen. The star represents statistically significant differences.

Discussion

The infusion of THAM in patients with chronic alveolar hypoventilation
used the development of a relative metabolic alkalosis superimposed on the
e-existing respiratory acidosis. It was possible to correct the *p*H in all
tients with uncompensated respiratory acidosis. However the infusion of
HAM caused in some patients an accentuation of the pre-existing anoxemia
at became very severe. The development of this severe anoxemia is con-
lered hazardous; hence THAM cannot be considered a therapeutic tool in
spiratory acidosis unless other means of oxygenation are applied.[3,4] Oxygen

breathing is unsuccessful in this respect since it deprives the organism of a important respiratory stimulant. Hence during oxygen breathing and THA administration further alveolar hypoventilation developed. The combinati of a respiratory stimulant (Prethcamid) and THAM while the patients we receiving oxygen resulted in a clear improvement of alveolar ventilation only one patient; the others showed either a slight effect or no effect at all.

It appears from these data that mechanical aids are necessary to mainta alveolar ventilation if THAM is given to correct the acidotic pH in chron alveolar hypoventilation.[5]

While no desirable therapeutic efficacy of THAM administration in patien with respiratory acidosis could be demonstrated, the investigations provi some insight into the respiratory regulation in patients with chronic respir tory acidosis. Patients with uncompensated respiratory acidosis decrease their arterial carbon dioxide tension during THAM infusion. On the oth hand, patients with compensated respiratory acidosis developed alkalotic p values during THAM infusion and showed a rise in carbon dioxide tensio These results indicate that chronic emphysematous patients adjust the ventilation to find a new equilibrium between the stimulus provided by t acid pH and the one exerted by the low oxygen tension. It seems that lo oxygen tension acting in concert with an acid pH results in a decrease in alve lar ventilation smaller than that observed in patients with a low oxygen tensi and an alkaline pH. Thus Group I shows a fall in oxygen tension as well a small decrease in carbon dioxide tension, while Group II shows a fall of oxyge tension but a rise in carbon dioxide tension. In both groups a fall in respir tory quotient has to be postulated as it was found in the experiments in norm individuals.

Summary

THAM (2-amino-2-hydroxymethyl-1,3-propanediol) was given to patients with chronic alveolar hypoventilation and respiratory acidosis. T infusion provoked, as in normal individuals, respiratory regulatory change notably alveolar hypoventilation with marked anoxemia. Since these chang are contrary to the therapeutic end points, THAM cannot be recommend for the management of chronic respiratory acidosis.

Acknowledgements

I acknowledge the aid and assistance of Donald J. Massaro, Emil F. Misko ski, Hall Canter, Francis S. Pooser, and George Tralka.

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THE USEFULNESS OF THAM IN METABOLIC ACIDOSIS*

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t is well appreciated, we believe, that the use of buffers in problems of dosis is in a sense analogous to the use of aspirin in reducing the metabolic ards of the pyrectic state. In the latter instance the cause of the fever is ignored in the physician's therapeutic regimen, but the dangers of its tinuation are appreciated. Similarly it is now recognized that during the dotic state various metabolic alterations in the body produce a refractoriness standard forms of treatment. It is the purpose of the administration of AM to restore a more favorable internal environment for the action of these gs. The return of the pH to normal by the addition of buffer is admittedly emporary reprieve unless circulatory, endocrine, and respiratory abnormali- are dealt with simultaneously.

t has been our custom to compute the dosage of THAM necessary to restore pH to normal at the particular reading of $p\text{CO}_2$ and HCO_3 present in the ient, to give this amount rapidly, and to repeat the procedure with an addi- nal dosage being calculated on the new readings of $p\text{CO}_2$ and HCO_3 after initial administration. If there is little or no response by the patient to definitive treatment of the cause of the acidosis *during* this temporary pH toration, the prognosis for survival is admittedly poor. In other words the of THAM for "cure" is not our concept of the value of this drug. Rather, great value lies in giving the patient and his physician a short opportunity rely on more physiological measures, and in *temporarily* removing the patient m a refractory state.

ase 1. Our first experience with the use of THAM in metabolic acidosis s somewhat favorable. We saw in this patient the development of acute tress of sudden metabolic acidosis and were able to relieve his symptoms for o 3 hours.

This 58-year-old white male had his venous blood sampled for analysis 15 n. before and 15 min. after an attack of coronary occlusion and septal infarc- n, which occurred as he was being given anesthesia. He was in shock and s given Neo-synephrine and oxygen. The bicarbonate level dropped from to 20 mEq.

The patient was well enough to be transferred to the recovery room shortly reafter. Four hours later he was gasping for breath. A venous blood nple was taken that showed a pH of 7.06, an HCO_3 of 18, and a $p\text{CO}_2$ of The patient's oxygen saturation was 92 per cent. An arterial sample wed the pH to be 7.25, the HCO_3 was 17, and the $p\text{CO}_2$ was 42. A dosage 32 mEq. of THAM was given. This did not markedly improve the patient's d-base condition but he calmed down, went to sleep, and awakened in 1 ir, quite comfortable. He spoke rationally with his relatives.

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* The work described in this paper was supported in part by The District of Columbia erculosis Association, Washington, D.C., and by Research Grant H-4783 from the Na- al Heart Institute, Public Health Service, Bethesda, Md.

The patient had a somewhat rougher time for the next 2 days, but Nec synephrine was discontinued on the third postanesthetic day. The bicarbonat remained at 18 mEq. The patient has recovered.

We should have treated him continuously with the buffer to reduce the wor of hyperventilation in the presence of a marked circulatory defect. It wa not possible to retard the rate of Neo-synephrine administration without de pressing the blood pressure while THAM was being given.

We have since successfully corrected in four patients episodes of metaboli acidosis with the conventional dosage of THAM buffer. One of these patient died during a second episode of metabolic acidosis.

Case 2. This Negro female (FIGURE 1) was studied quite carefully befor operation because she was 106 years old and needed a leg amputation for prc gressive septic gangrene that had affected her left foot for 3 months. He kidney concentration was only fair. She had fairly well compensated for metabolic acidosis prior to amputation, but we are sure some physicians woul have tried to improve the bicarbonate level before surgery, since the HCC ranged between 16 and 19 mEq.

The patient did fairly well for 30 hours after the amputation until we inte fered with her metabolism by administering a pint of blood over a 4-hour perio because of anemia and hypoproteinemia. She promptly became hypertensiv anuric, and dyspneic, and appeared to have pulmonary edema.

The arterial blood showed a pH of 7.41, an HCO_3 of 5.8, and a pCO_2 of 1 THAM (116 mEq.) was given over a 70-min. period. The arterial blood the revealed a pH of 7.41, and HCO_3 of 17.5, and a pCO_2 of 28. The urinary outpu returned; blood pressure dropped from 150/90 to 100/50; and respiration de creased from 40 to 30. The next morning arterial pH was 7.56, the HCC was 20.4, and the pCO_2 was 23.5. The patient did well until the sixth day afte operation when suddenly she went into irreversible shock. A bicarbonate leve of 10 mEq. could not be corrected with 250 mEq. of THAM—twice the cor ventional dosage—and she died. The cause of death was pulmonary embc lism.

Methods

Arterial samples taken in heparinized syringes have been found necessar during the observation of metabolic acidosis. Both semimicro and micr Astrup techniques were employed for the determination of blood pH, bica bonate, and pCO_2. Oxygen saturation was measured by the Waters ea oximeter.

The dosage of THAM given in 0.3 M solution was calculated from the b carbonate deficit as follows:

$$0.30 \times \text{wt. in kg.} \times (23 - \text{HCO}_3)$$

Failure of correction and death followed the above regimen in 2 instance (1) heart failure from pulmonary embolization (Case 2); and (2) heart failu from carcinomatous pericarditis, Dicumerol, and cardiac tamponade.

Immediate permanent correction of the metabolic acidosis occurred in:

se of shock from inadequate operative blood replacement; 1 case of shock llowing cardiac arrest and resuscitation; 1 late postoperative temporary isode of heart failure; and 1 case of pulmonary edema following transfusion ase 2).

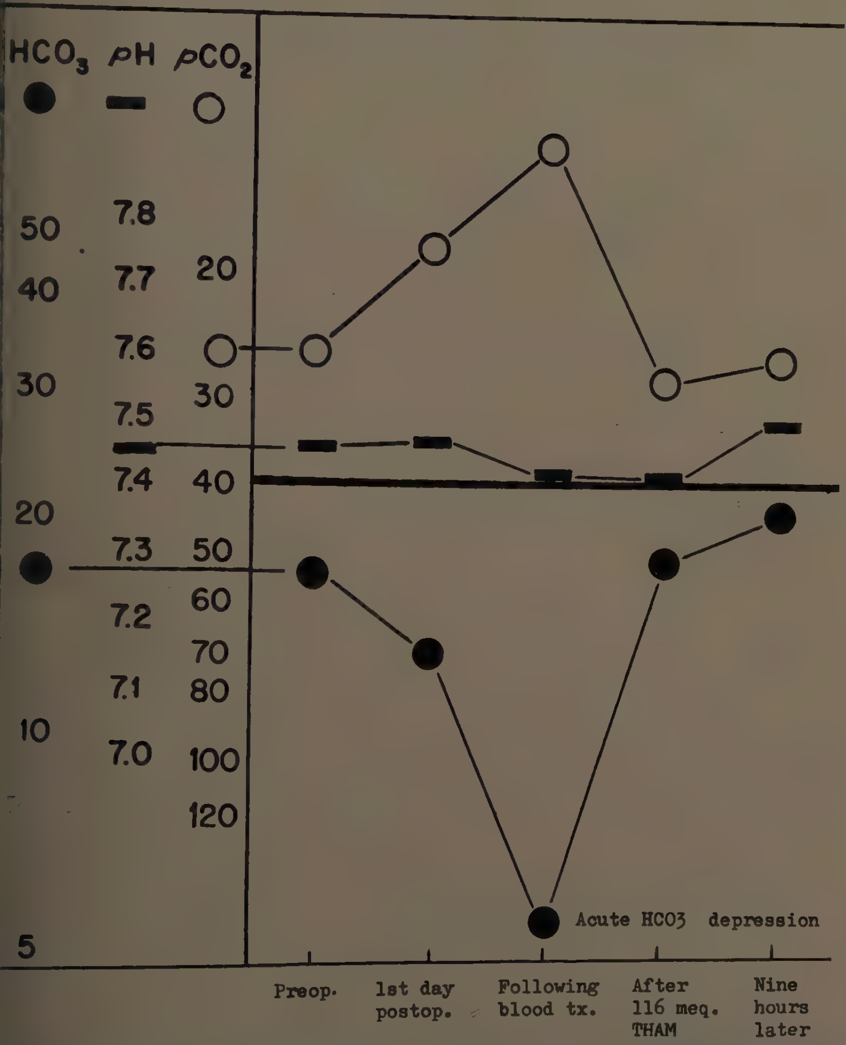

Figure 1 (Case 2). Graph illustrating the response of an acute bicarbonate depression to mEq. of THAM. Arterial blood values were obtained by the micro-Astrup technique. e patient's weight was estimated at 30 kg. A modified Singer-Hastings nomogram is on left. Normal values are indicated by the central horizontal line.

Conclusion

From our experience it seems that one should treat metabolic acidosis chemi- ly as it appears, rather than depend upon respiratory and renal compensation in these cases with circulatory failure. The use of THAM affords more tin to employ the accepted therapeutic measures directed toward the cause of the episodes.

Further experience with the prompt use of buffers (such as THAM) wh metabolic acidosis is detected may make the chemical treatment of this conc tion more acceptable.

ME *IN VIVO* AND *IN VITRO* OBSERVATIONS ON THE EFFECTS OF TRIS(HYDROXYMETHYL)AMINOMETHANE IN DIABETIC ACIDOSIS*

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Introduction

n the course of collaborative tris(hydroxymethyl)aminomethane studies h Merrill's group reported elsewhere in this monograph we noted that betic patients with renal acidosis developed a more severe hypoglycemia n acidotic nondiabetic patients. This report will illustrate the effective ninistration of nontoxic doses of tris(hydroxymethyl)aminomethane (Tris fer or THAM) to acidotic diabetic patients.

ris buffer will be shown by *in vitro* electrophoretic techniques to release ulin normally bound to globulins in the sera of treated and insulin resistant betics.

Material and Methods

roup I. Six patients from a series of 168 decompensated acidotic diabetic ients admitted to the New England Deaconess Hospital, Boston, Mass., re selected for a preliminary trial of Tris buffer (THAM) supplied by Abbott boratories, Chicago, Ill. These patients were unable to tolerate conserva- e sodium bicarbonate or lactate therapy because of severe cardiac and/or lium-retaining renal failure.

aboratory studies including those of arterial *p*H and total CO_2 were per- med in the Baker Clinic Research Laboratory and Department of Clinical thology, New England Deaconess Hospital.

ost-mortem examinations were performed by the Department of Pathology, w England Deaconess Hospital, and we are indebted to Merle A. Legg who efully reviewed renal sections with us for any evidence of lesions described Brinkman elsewhere in these pages.

roup II. A representative case, M.C., of uncomplicated diabetic acidosis ne of a controlled series currently under investigation to determine whether mpt use of nontoxic doses of THAM plus saline and insulin offers any clinical antages over conservative therapy (Joslin *et al.*, 1959).

aper electrophoresis of serum equilibrated with I^{131}-labeled insulin was formed with both veronal and tris-maleate buffers, *p*H 8.6 in a Spinco-Dur- n cell.

x insulin was iodinated† and resuspended in 1 per cent human albumin with nal specific activity of 7 millicuries (mc.) per mg. insulin.‡ Twenty lambda erum equilibrated for 30 min. at room temperature with 0.04 μc. of labeled

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This investigation was supported in part by Research Grant 2A-5070 from the National itute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

By the Abbott Laboratories, North Chicago, Ill.

This material was kindly prepared and supplied to us by Marvin Mitchell of the Isotope t, Lemuel Shattuck Hospital, Boston, Mass.

insulin, was applied to Whatman 3.0 × 30.6-cm. paper strips previou
saturated with crystalline insulin.* Sidney Ingbar kindly provided I
labeled thyroxin (T_4) for a comparison of T_4 and labeled insulin distribution
veronal and Tris-maleate buffer systems as outlined by Ingbar and Freinl
(1960). I^{131} distribution was assayed by scanning dried paper with a Nucle
Chicago strip counter prior to protein staining with bromphenol blue. Prote
distribution was determined with a Beckman model RA Analytrol employi
appropriate interference filters.

Results and Discussion

Group I. Results *in vivo* of the effects of 0.3 *M* Tris buffer (THAM)
ministered at a dose of 0.5 to 1.0 mM/kg./hour for a total dose of 3.8 to
mM/kg., are summarized in TABLES 1 to 3. Each patient received 1 gm.
calcium gluconate per 0.1 unit rise in *p*H to prevent tetany.

TABLE 1

USE OF THAM IN DIABETIC ACIDOSIS COMPLICATED BY CARDIAC AND/OR RENAL FAILU

Group I: Clinical data

Pt.	Wt. (kg.)	Duration (yr.)	Age (yr.)	BUN (mg. %)	Na (mEq./l.)	K (mEq./l.)	Cl (mEq./l.)	Before THAM CO_2 (mEq./l.)	Before THAM *p*H	Before THAM B.S. (mg. %)	After THAM CO_2 (mEq./l.)	After THAM *p*H	After THAM B.S. (mg. %)	Insul. units
G.S.	72	14	31	82	129	6.8	107	6.0	6.92	680	32	7.32	82	140
A.K.	71	18	32	118	134	5.4	90	8.4	7.16	758	29	7.35	90	20
M.T.	80	10	79	41	125	6.4	93	13.0	7.15	590	26	7.30	110	30
E.A.	66	20	69	130	145	4.4	101	12.1	7.16	625	23	7.31	228	40
E.R	65	21	47	240	138	4.9	90	9.0	7.13	560	25	7.30	130	10
T.F.	68	13	42	90	146	4.5	100	4.0	7.17	1238	26	7.40	200	1600

These subjects were considered preterminal diabetics in acidosis because
underlying cardiovascular-renal failure. All patients except M.T. of Grou
had clinical evidence of azotemia for 2 to 8 years prior to their terminal
mission. All patients had clinical evidence of heart disease before this termi
episode of acidosis.

TABLE 1 summarizes the age, weight, duration of diabetes, arterial *p*H, C
electrolytes in venous blood, blood urea nitrogen, and total insulin and T
buffer doses required to normalize *p*H, CO_2, and blood sugar. Only 2 of th
patients (G.S. and T.F.) required 600 mM of Tris buffer to normalize *p*H. C
of these, T.F., was in profound shock and anuric despite treatment w
Levophed and digitalis during this terminal episode of diabetic acidosis.
tient T.F., in contrast to our 5 other patients, failed to demonstrate reversa
insulin resistance and continued to develop progressive hyperkalemia desp
correction of his diabetic acidosis with 1600 units of insulin. All other subje
in TABLE 1 had low insulin requirements (less than 50 units in 4 subjects)
spite severe acidosis. This must be in part related to the effective bufferin

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* Squibb Products, New York, N.Y.

ess hydrogen ion by THAM throughout the entire total body water. How-r, terminal uremia per se is associated with reduction of insulin require-nts (Joslin *et al.*, 1959), and the organic amines of uremia may share some of hypoglycemic properties of Tris buffer.

ABLE 2 (case G.S. of Group I) illustrates the effect of THAM on hypogly-ic response to circulating insulin (140 units) given 6 hours before 0.3 *M* AM was started. A slow infusion of 1.0 mM/kg./hour from the sixth to h hour, produced significant reduction of blood sugar from 680 to 32 mg. cent and lowering of serum K from 6.8 to 4.0 mEq/l. with only partial ection of acidosis from 6.92 to 7.09. However, in light of Robin's study in

TABLE 2

EXAMPLE OF THAM REVERSAL OF INSULIN RESISTANCE

Case G. S.: Group I

ne rs)	B.S. (mg. %)	Venous			Arterial		BUN (mg. %)	Therapy and course
		Na (mEq./l.)	K (mEq./l.)	Cl (mEq./l.)	pH	CO_2 (mM/l.)		
	975	116	7.9	97	6.80	4.0	82	140 U. crystalline insulin, 500 cc. hypertonic lactate saline with no improvement
	680	129	6.8	107	6.92	6.0		4-hour infusion of 0.3 M THAM with simultaneous clinical improvement and hypoglycemic reaction when pH recorded 7.09 CHO—20 gm./hr. required to maintain normal blood sugar
	32	132	4.0	110	7.09	10.0		
	119	133	3.2	106	7.13	15		CHO reduced to 10 gm./hr. plus 6-hour infusion 0.3 *M* THAM plus 50 mEq. KCl
	82	132	3.8	99	7.32	32	78	Oliguric regimen—500 cc. of 50 per cent D&W/day with no further insulin requirements

s reported elsewhere in these pages Tris buffer or THAM may be effec-ly buffering intracellular acidosis and facilitating both net cellular dextrose zation and potassium accumulation before extracellular or arterial pH is nalized. Additonal *in vivo* and *in vitro* experiments employing amine buff-nd indicators of cellular pH are essential to determine the effects of Tris er upon cellular acidosis. The clinical observation of increased sensitivity irculating insulin warrants special caution and monitoring of both blood r and potassium during infusions of Tris buffer in diabetic subjects.

ABLE 3 summarizes the clinical courses and causes of death in our 6 pa-ts of Group I. Despite correction of acidosis with THAM, all patients umbed to their underlying cardiovascular-renal disease. Two patients . and T.F.) showed no improvement in their underlying uremic coma with restoration of normal acid-base and electrolyte balance. Both A.K. an T.F. demonstrated acute tubular necrosis superimposed upon diabetic nephrop athy consistent with their terminal hypotension. Post-mortem was r stricted to the abdomen in A.K., but T.F.'s irreversible central nervous syste signs were attributed to multiple foci of cerebral necrosis secondary to shoc and advanced cerebral arteriolarsclerosis.

Only one patient, G.S., had a transient diuresis, but no sustained improv ment in renal function was observed; he expired seven days after correction acidosis with diabetic nephropathy complicated by cardiac failure and bronch pneumonia.

TABLE 3

GROUP I: CLINICAL COURSE AND POST-MORTEM FINDINGS IN SIX DIABETICS WITH PRETERMINAL ACIDOSIS RECEIVING THAM

Pt.	Clinical course	Days after THAM	Cause of death
G.S.	Transient diuresis post THAM but cardiorenal failure refractory to digoxin and fluid restriction.	7	Diabetic nephropathy with u mia, congestive heart failu and bronchopneumonia.
A.K.	Terminal uremic coma and anuria—not improved with correction of acidosis.	12	Diabetic nephropathy and acu tubular necrosis with uremia.
M.T.	Oliguria and congestive failure refractory to digoxin and fluid restriction—no diuresis.	6	Acute anteroseptal infarction—d betic nephropathy with so hydropic degeneration of pro imal tubule.
E.A.	Clinical improvement until massive GI bleeding exceeded blood replacement.	1	Diabetic nephropathy with urem acute hemorrhagic ulcerative g tritis—arteriosclerotic heart d ease, coronary atherosclerosis.
E.R.	Terminal uremia with no clinical improvement despite correction of acidosis.	4	Diabetic nephropathy with urem bronchopneumonia, arterioscle tic heart disease.
T.F.	Pt. had no clinical improvement and required 1600 units of insulin. Anuria and shock did not respond to lanatoside-c or levophed in terminal state.	1	Diabetic nephropathy and acu tubular necrosis—multiple foci cerebral necrosis secondary shock and advanced arteriol sclerosis.

Patient E.A. succumbed to massive gastrointestinal bleeding secondary to ulcerative gastritis and uremia with diabetic nephropathy.

The Department of Pathology found equivocal evidence of proximal tubu hydropic degeneration in one patient, M.T., who expired 6 days after receivi THAM. This has been observed with comparable frequency among patie not receiving THAM who have had a prolonged osmotic diuresis secondary poorly controlled diabetes. It must be noted, however, that our 6 patie receiving THAM were complicated by underlying diabetic nephropathy a in two instances, acute tubular necrosis secondary to shock. Hydropic generation could be obscured by the extensive renal pathology demonstrated our patients in Group I. Brinkman's terminal case of diabetic acidosis dem strating hydropic degeneration, received 600 mM of THAM over a brief 1-

-hour period without conservative replacement of salt, water, or insulin deficits rior to correction of acidosis. We have employed THAM at lower doses with ower rates of infusion as an adjunct to conservative therapy and have possibly voided the serious toxicity suggested by Brinkman elsewhere in this monoraph.

More extensive clinical trials of Tris buffer in uncomplicated cases of diabetic cidosis are in progress. To date, 4 adult patients admitted in decompensated iabetic ketoacidosis have received 40 to 100 units of clear insulin promptly by ein. These patients were rehydrated during the first 4 to 6 hours with 3 to 4 of a 0.1 *M* saline-0.1 *M* THAM solution. A representative case, M.C. TABLE 4), with diabetes of 15 years' duration, was admitted with diabetic cidosis secondary to overeating and insufficient insulin. She had no clinical vidence of cardiac or renal disease and only transient oliguria, and azotemia ttributed to dehydration during her first few hours of admission. This paent's clinical and laboratory data are summarized in TABLE 4 and indicate

TABLE 4

USE OF THAM IN UNCOMPLICATED DECOMPENSATED DIABETIC KETOACIDOSIS

Group II: e.g., Patient M.C.

Time hours)	B.S. (mg. %)	Arterial		Therapy
		pH	CO_2 (mEq./l.)	
0	575	7.20	10.2	90 U. of crystalline insulin, 1 l. of 0.15 *M* saline, 1 l. of 0.1 *M* saline and 0.1 *M* THAM
3	320	7.32	21.0	2 l. of 0.1 *M* saline and 0.1 *M* THAM
6	181	7.40	28.1	10 gm. CHO, 5 mEq. potassium p.o. per hour × 6.

rompt reversal of diabetic acidosis and prerenal-azotemia without untoward eactions to THAM. Our preliminary series of four cases of uncomplicated iabetic acidosis treated with THAM have recovered with less than 100 units regular insulin. A large carefully controlled and studied series will be necssary before we prove our clinical impression that nontoxic doses of (THAM) n buffer cellular acidosis and reduce the insulin requirements for management diabetic coma.

Representative paper electrophoretic data obtained as outlined in the meth- ls section, are summarized in FIGURES 1 to 4. In all figures, the dotted line ove the paper strip represents the relative distribution of the labeled hormone. he solid line below each strip represents the distribution of the major protein actions.

FIGURE 1 confirms the data of Ingbar and Freinkel (1960) with paper satu- ted with insulin. Forty-three per cent of thyroxin (T_4) is shown to migrate ead of albumin in the Tris-maleate buffer, while no migration occurs in this gion in a veronal buffer. Hamolsky and Stein have summarized elsewhere this monograph the difficulties in evaluating the physiological significance of is data. We have illustrated the T_4 Tris-maleate distribution in order to

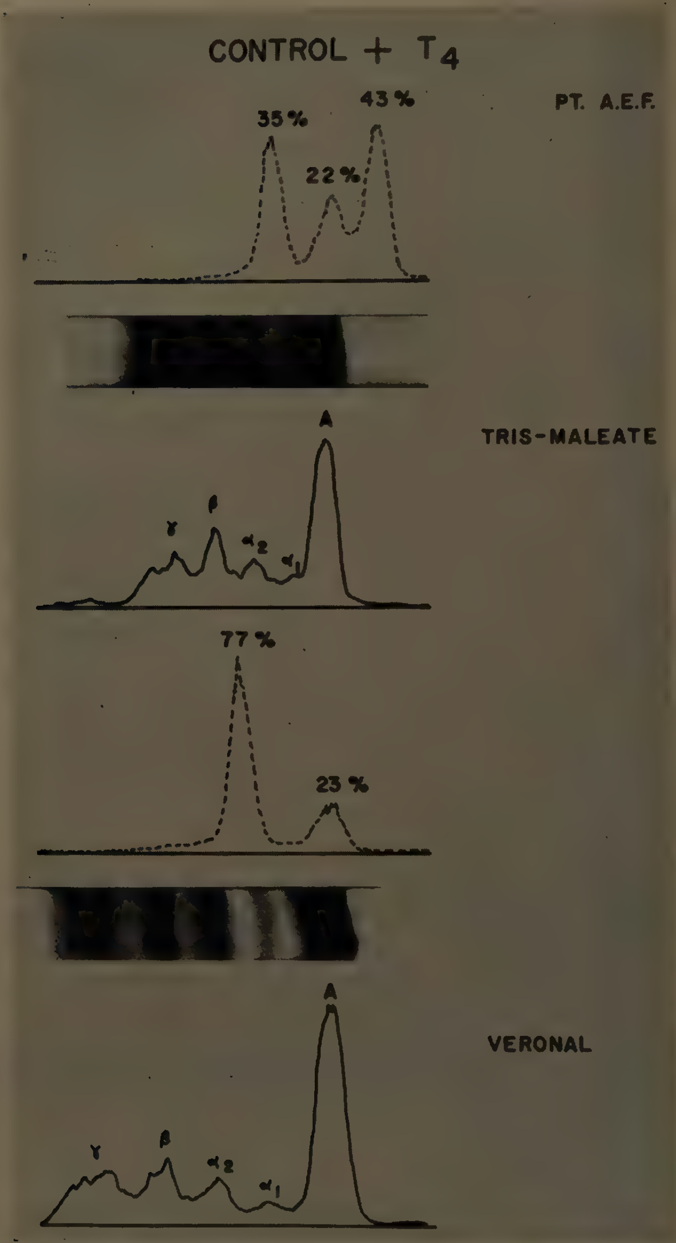

FIGURE 1. Control serum equilibrated with radioactive thyroxin (T_4). Electrophor
with Tris-maleate buffer demonstrates prealbumin thyroxin binding not observed with vero

calize prealbumin as a T_4 binding prealbumin complex because prealbumin rmally exists in concentrations too low to be detected by protein stains.

In FIGURE 2 a control serum equilibrated with labeled insulin and electrohoresed as summarized in the methods section illustrates a predominant peak activity in the alpha 1-albumin area in both Tris-maleate and veronal buffer

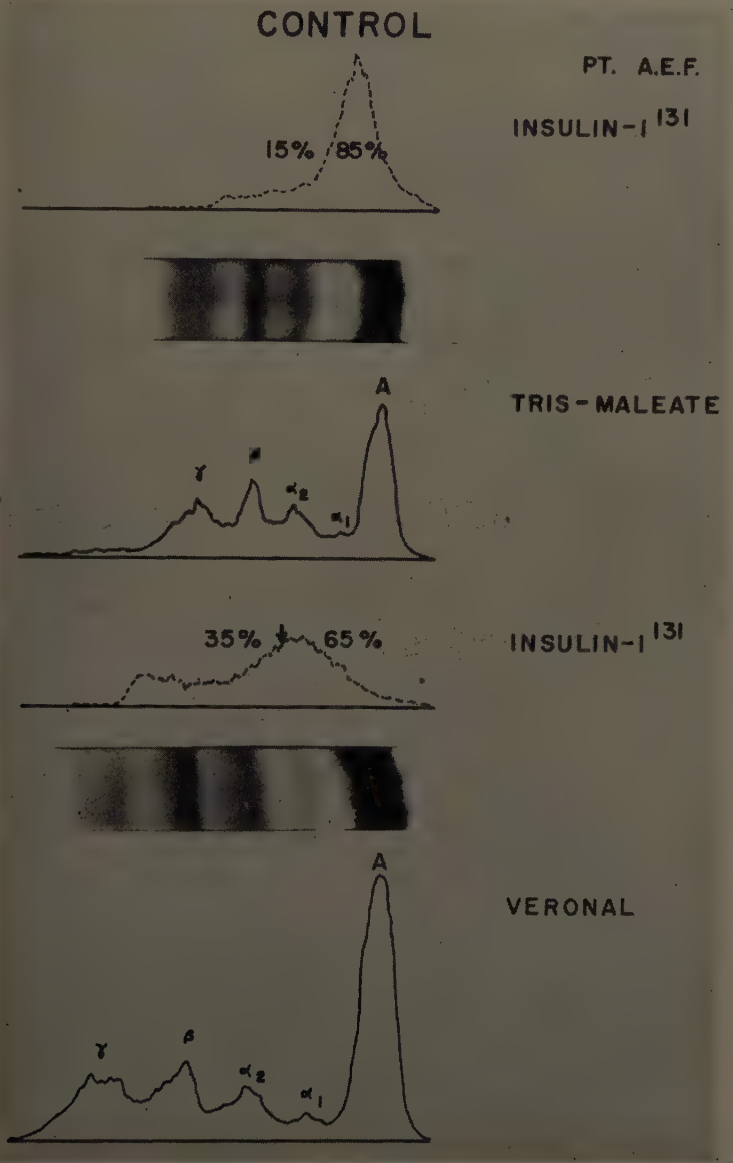

FIGURE 2. Control serum equilibrated with radioactive insulin demonstrates major localtion of activity in the alpha 1-albumin zone with both Tris-maleate and veronal.

systems. Randle and Taylor (1958) and Antoniades *et al.* (1958) have show that free insulin determined by bioassay migrates in the alpha 1-albumin zon that corresponds to our area of peak I^{131} insulin activity. Labeled insulin i contrast to thyroxin (T_4) shows no evidence of migration with the prealbumi zone in Tris buffer (FIGURE 1).

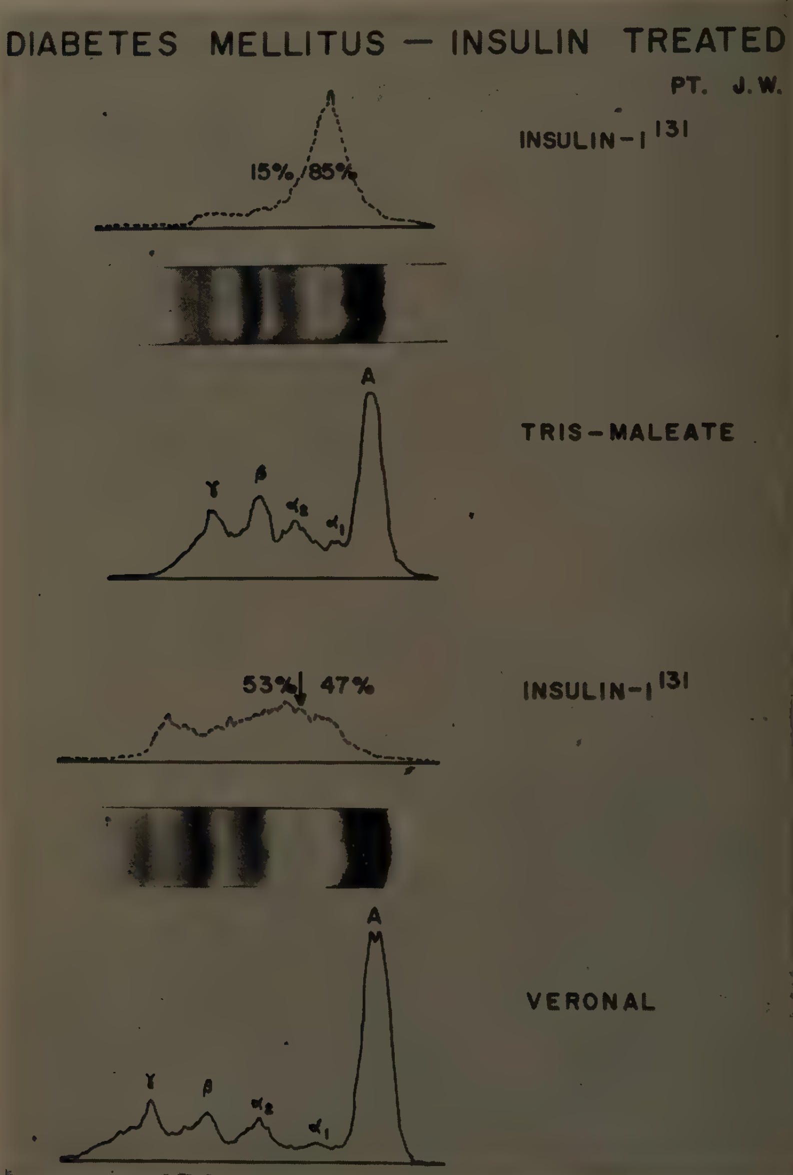

FIGURE 3. Serum from insulin-treated diabetic demonstrates 47 per cent of activity alpha 1-albumin zone with veronal and 85 per cent of activity in alpha 1-albumin zone w Tris-maleate.

FIGURES 3 and 4 illustrate an identical alpha 1-albumin peak of insulin
tivity in the Tris-maleate system in an insulin-treated diabetic (30 to 40
its/day) and a mild insulin-resistant patient requiring 150 to 200 units/day.
he distribution of insulin activity in the veronal system demonstrates a de-

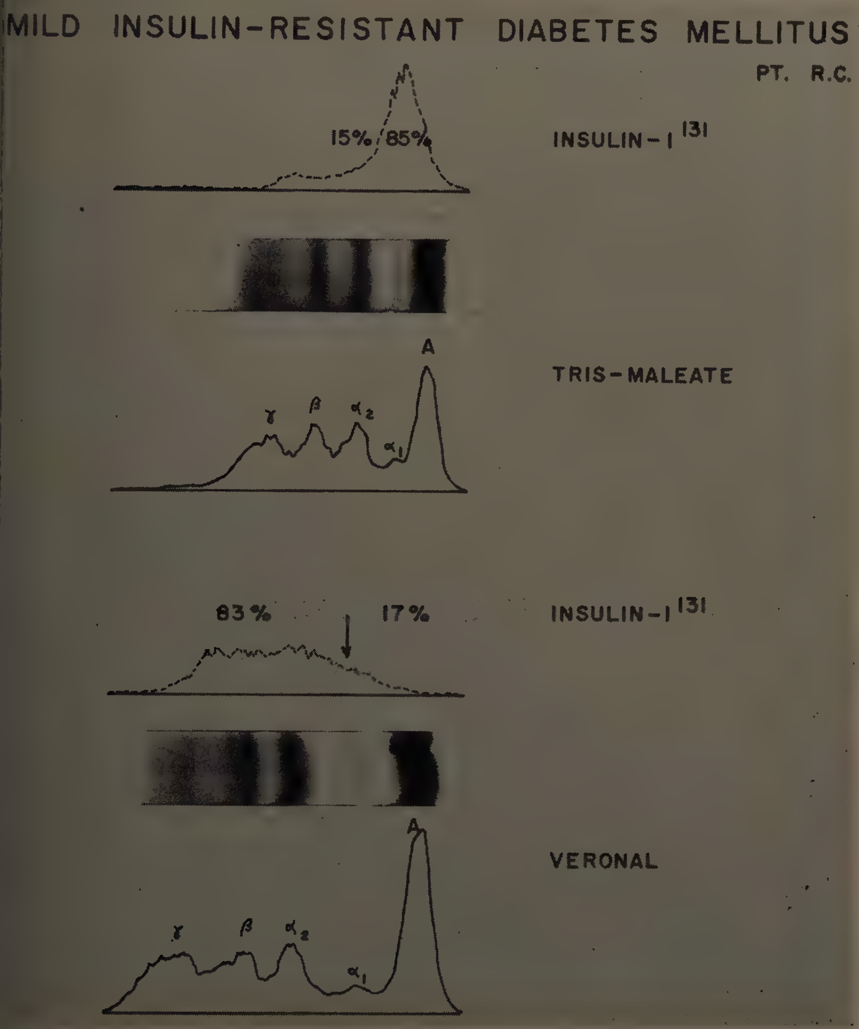

FIGURE 4. Serum from insulin-resistant diabetic demonstrates only 17 per cent of activity
lpha 1-albumin zone with veronal and 85 per cent of activity in alpha 1-albumin zone with
is-maleate.

ased distribution of labeled insulin in the alpha 1-albumin zone and increased
tivity in the globulin area. The veronal system illustrates a progressive
crease in beta and gamma globulin distribution of insulin activity as a func-
n of insulin resistance. This is demonstrated in the sera from a control (35

per cent), an insulin-treated (53 per cent), and an insulin-resistant (83 per cent patient (FIGURES 2 to 4 respectively). Berson and Yalow (1957) have show similar results with more elaborate preparative techniques and concluded tha insulin-binding antibodies migrate in the beta-gamma globulin zone. Our *i vitro* results suggest that a Tris-maleate buffer dissociates beta-gamma globulin bound insulin and increases "free" insulin migrating in the alpha 1-albumi area. Antoniades (1958) has shown that the dissociation of serum insuli basic protein complexes increase with increasing *p*H, and our data sugges that a Tris-maleate buffer can release insulin bound to globulins *in vitro*.

Summary and Conclusions

(1) Tris buffer (THAM) has been administered to 6 preterminal diabet patients with decompensated metabolic acidosis associated with ketoacidos and cardiorenal failure. THAM (4 to 9 mM/kg.) slowly administered over 4- to 12-hour period corrected the acidosis in all cases and increased sensitivit to circulating insulin in 5 of the 6 patients. These 6 patients eventually su cumbed to their underlying cardiorenal failure, but post-mortem examinatio revealed no microscopic evidence that THAM contributed to cause of death.

(2) A preliminary trial of Tris buffer (THAM) as an adjunct to conservati therapy in 4 patients with uncomplicated diabetic acidosis is described. carefully controlled and extended series will be necessary before we can pro our clinical impression that the addition of THAM to conservative therapy ca reduce insulin requirements and provide a more rapid reversal of cellular ac dosis in diabetic coma.

(3) Paper electrophoresis studies *in vitro* of serum from insulin-treated a resistant subjects, suggest that Tris-maleate buffer releases insulin normal bound to globulins at a *p*H of 8.6 in other buffer systems. This represen another biological system in which Tris does not function as an inert buffe but these interactions of Tris with proteins could be of clinical significanc

(4) The physical chemical interactions of the amine buffers with biologic systems will require more extensive *in vivo* and *in vitro* study before they can recommended for general clinical use. The amine buffers promise to be exciti research tools for further study of the pathologic physiology of diabetes mellit

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FFECTS OF 2-AMINO-1-HYDROXYMETHYL-1,3-PROPANEDIOL (TRIS) DURING CARDIAC BYPASS PROCEDURES*

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Introduction

n a well-conducted, routine cardiopulmonary bypass, serious acidosis uld not occur and, therefore, antacids should rarely be necessary. Because re are certain dangers inherent in the administration of antacids, they should used only when acidosis has reached serious proportions and shown evidence continued progression. This paper will attempt to explain circumstances ly to result in serious acidosis, methods for rapid detection and quantita- of the acidosis, and a reasonable formula for determining therapy.

Causes and Types of Acidosis

cidosis is produced by the accumulation of acid products of metabolism the blood stream, tissue fluid, and probably intracellularly, or by the ad- istration of acids. The most important metabolic acid is carbon dioxide carbonic acid. Because carbonic acid is eliminated by the respiratory te, it is generally and incorrectly not considered to be a metabolic acid. clarity, I shall divide metabolic acids into respiratory-dependent acids rbonic acid) and nonrespiratory-dependent acids and then refer to them ply as respiratory and nonrespiratory acids. The latter include inter- diary organic acids normally oxidized to carbon dioxide and water and also rganic acids normally excreted by the renal route; I am not concerned with latter group in acute acidosis occurring during or after cardiopulmonary ass.

espiratory acidosis is uncommon during cardiopulmonary bypass as most ficial lungs eliminate carbon dioxide efficiently. It may occur, however, h improper use of the small bubble oxygenator or the membrane lung and se reversible central nervous system depression. In the postbypass period iratory acidosis may occur if there is central nervous system depression n anesthesia, hypothermia, metabolic acidosis, or if there has been lung age. Unrecognized, this may result in serious central nervous system, ulatory, and renal depression.

onrespiratory acidosis is a gross measure of tissue oxygen debt and, con- uently, an index of the adequacy of tissue perfusion.[7,9] A progressive ease in nonrespiratory acids is therefore an indication of circulatory de- pensation. Slight acidosis results from the circulatory depression of eral anesthesia and may increase during cardiopulmonary bypass if the fusion rate is inadequate. The addition of large quantities of unbuffered citrate blood also results in nonrespiratory acidosis. Progressive acidosis

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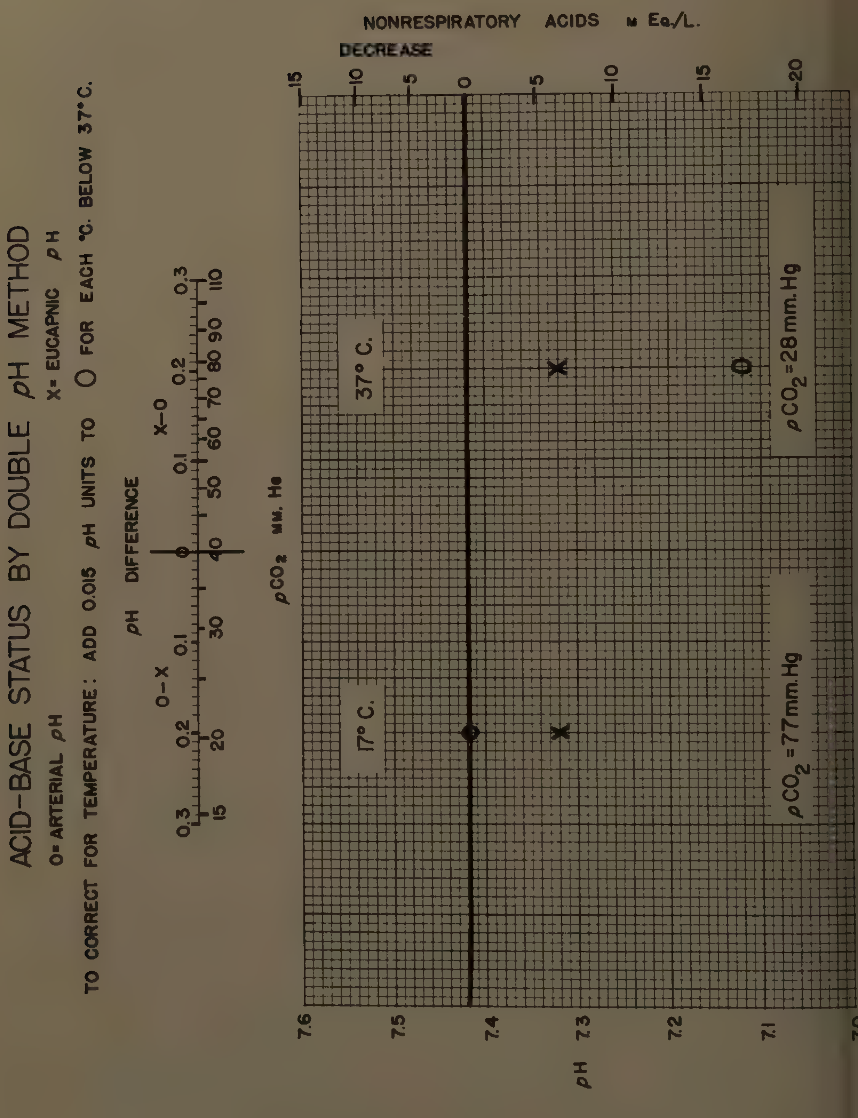

ACID–BASE STATUS BY DOUBLE pH METHOD
O= ARTERIAL pH
X= EUCAPNIC pH
TO CORRECT FOR TEMPERATURE: ADD 0.015 pH UNITS TO O FOR EACH °C. BELOW 37° C.
pH DIFFERENCE
O–X
X–O
0.3
0.2
0.1
0
0.1
0.2
0.3
15
20
30
40
50
60
70
80
90
110
pCO2 MM. Hg
NONRESPIRATORY ACIDS mEq./L.
DECREASE
15
10
5
0
5
10
15
20
37° C.
17° C.
pCO2 = 28 mm.Hg
pCO2 = 77 mm.Hg
pH
7.6
7.5
7.4
7.3
7.2
7.1

kely to occur during perfusion when it is unduly prolonged or when there xtra oxygen demand, as following total circulatory arrest in hypothermia or n warming from the hypothermic state has been too rapid in relationship nyocardial function or available blood volume. Since the actual period of usion is likely to be well planned and relatively short, acidosis is more ne to occur in the immediate postperfusion period. Causes of circulatory ompensation that may result in nonrespiratory acidosis include: myocardial ression due to ventriculotomy, circulatory arrest, or chemical cardioplegia; o- or hypervolemia, heart block, increased pulmonary vascular resistance, respiratory acidosis. Such acidosis is dangerous because it aggravates ulatory and central nervous system depression and at best is slowly cor- ed.

Recognition of Acidosis

n short, straightforward cardiopulmonary bypass procedures, maintenance adequate ventilation, and occasional measurement of pH will probably ice to prevent trouble. A fairly good idea of circulatory status can be ained from periodic sampling of central mixed venous blood since in de- pensation the saturation will fall. Unfortunately, central samples are not ays easy to obtain, and the method is less applicable to deep hypothermia. culatory status may also be assessed by measurements of cardiac output or gen consumption, but this is generally impracticable. I have concluded t the easiest method of assessing respiratory and circulatory integrity is to isure acid-base changes, both during cardiopulmonary bypass and post- usion.

Acid-base Measurement

rom the limited standpoint of interest in preventing and treating acute l-base disturbances, it is sufficient to know any abnormality of hydrogen concentration produced by respiratory and nonrespiratory (circulatory) ors. The pH is the measure of the hydrogen ion concentration and, there- , contains this information. Any pH abnormality may be quantitated ply by determining what pCO$_2$ or what level of added acid is required to luce the same abnormality. The nonrespiratory abnormality is determined rendering an arterial blood sample eucapnic, that is, by bringing it to a

IGURE 1. Chart to plot acid-base changes during clinical cardiopulmonary bypass with ithout hypothermia. The nonrespiratory acid zero is equivalent to a buffer base of 49 ./l. Since only 1 line chart is used to determine the pCO$_2$, the pCO$_2$, except at 40 mm. must be considered only approximate. Below 40 mm. Hg the maximum error is 6 mm. and above 40 mm. Hg it is 13 mm. Hg. The chart is constructed from the nomogram of er and Hastings.[2] The assumption is made that the Rosenthal correction for pH change temperature is correct[10] and that it is equivalent to the correction for pCO$_2$ with change mperature. It is assumed that there is no change in the buffer base[2] "standard bicar- te,"[1] or eucapnic pH with change in temperature.[3] The chart is used as follows:

) Plot the arterial pH at any desired temperature.

) Plot either the eucapnic pH (the pH at 40 mm. Hg pCO$_2$), the "standard bicarbonate" rmality (the plasma bicarbonate abnormality at a pCO$_2$ of 40 mm. Hg), the buffer base rmality, or the pH difference resulting from a pCO$_2$ other than 40 mm. Hg (see line chart).

) The pH decompensation is the difference between the arterial pH and 7.42.

) The pCO$_2$ is determined by the difference between the arterial pH and the eucapnic This is correct for the temperature chosen for the arterial pH.

normal alveolar pCO_2 (40 mm. Hg). The respiratory abnormality is scribed by the difference between the pH of the eucapnic blood sample a that of the original arterial blood sample. These relationships are shown FIGURE 1.[5,6]

In hypothermia carbon dioxide becomes more soluble and also the dissoc tion constant changes, so that there are related changes in pCO_2 and pH. T relationship is a convenient one for, if the pCO_2 is kept constant, the pH d not change with temperature. This means that the buffer base of whole bl does not change with temperature nor does the eucapnic pH.[3] It is theref possible to use FIGURE 1 for determination of the respiratory and nonrespi tory pH factors (pCO_2 and buffer base) at any desired temperature, usu in blood-stream cooling, the esophageal temperature of the patient.

Correction of Acidosis in ACD Blood

FIGURE 2 illustrates the buffering of acidotic old bank blood by sodi bicarbonate. It is apparent that this cannot be accomplished in a closed c tainer but only when the release of carbon dioxide is possible. This anta can be used, therefore, only when respiratory function is adequate. The dition of Tris, however, produces correction of both the respiratory and nonrespiratory acidosis of a similar bottle of bank blood (FIGURE 3).[8] In ally, the buffering is facilitated slightly by increased respiratory action, after the blood has been brought to approximately pH 7.4, a normal respirat pCO_2 actually decreases the action of the buffer. Both FIGURES 2 an indicate that there are three basic components of the acidosis of the b blood. First there is a dilution acidosis, shown in FIGURE 4. A dilution 20 per cent reduces the eucapnic pH from 7.4 to 7.28. The addition the 880 mEq./l. of the trivalent citric acid reduces the buffer base an additi 13.8 mEq. and brings the pH to approximately 6.99. Metabolic acti during 25 days of banking at 4° C. produces about 5 mEq./l. of additi acid and reduces the eucapnic pH to about 6.8. A pH depression, theref below approximately pH 7.0 at the time the blood is drawn and pH 6.8 a prolonged banking is the result of CO_2 accumulation (TABLE 1). Since, administration of the blood, the respiratory acidosis can be corrected by sl hyperventilation and the dilution acidosis by redistribution of water, i necessary to correct only the nonrespiratory acidosis, which is the sum of citric acid and incomplete metabolism during banking. It is therefore rec mended that for fresh blood approximately 10 mEq. of antacid be administ for each 600-cc. unit; this need be increased only to about 15 mEq. for bl that has been banked for some time. Even if one desires to correct the resp tory acidosis as well, it is not necessary to use much additional Tris since high pCO_2 of the blood is chiefly the result of the nonrespiratory acidosis, the total carbon dioxide is only slightly above normal (TABLE 1 and FIGURE

Correction of Nonrespiratory Acidosis

As in the correction of the acidosis due to ACD blood administration, respiratory abnormality is relatively unimportant, provided that Tris is or there is ready egress for carbon dioxide. FIGURE 6 shows that whe

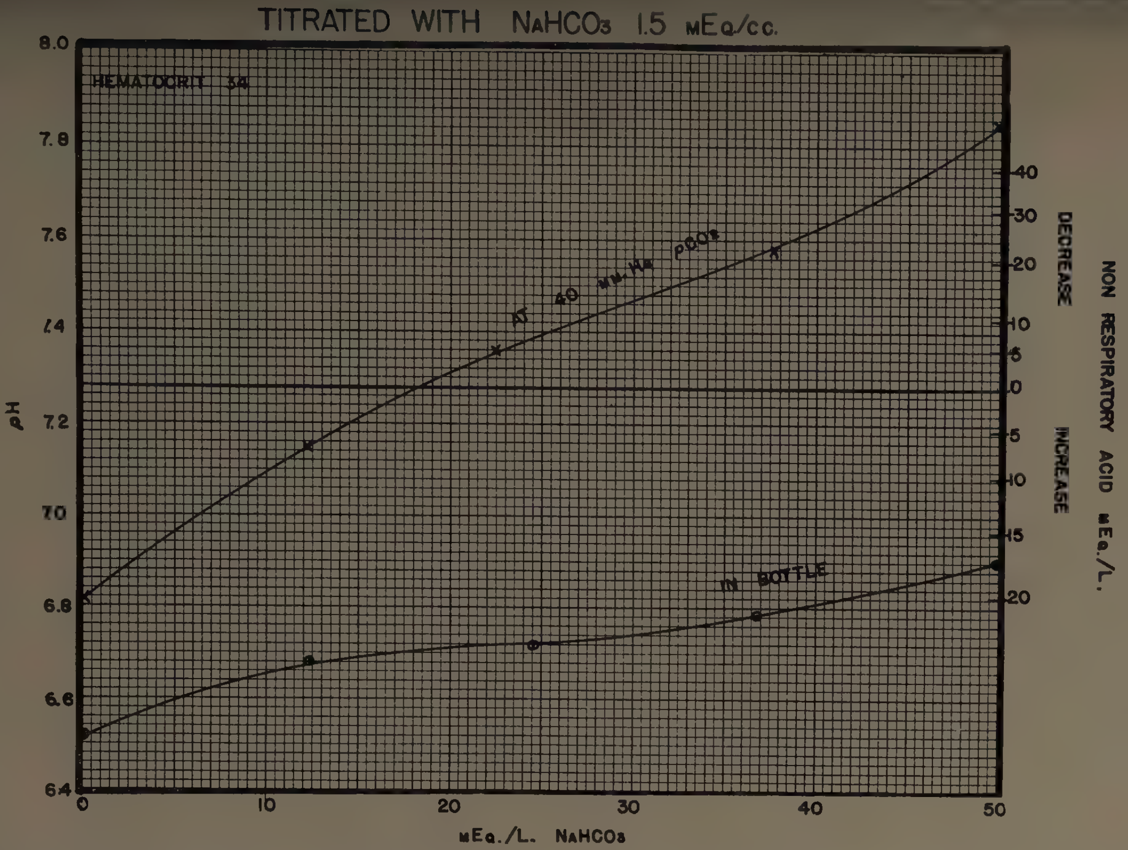


FIGURE 2. If sodium bicarbonate is added in a confined space, it has a very limited ability to raise the pH. If, while it is added, the $p\text{CO}_2$ is kept at a normal value of 40 mm. Hg, the pH is raised by the neutralization of fixed acids in approximately stoichiometric proportion.

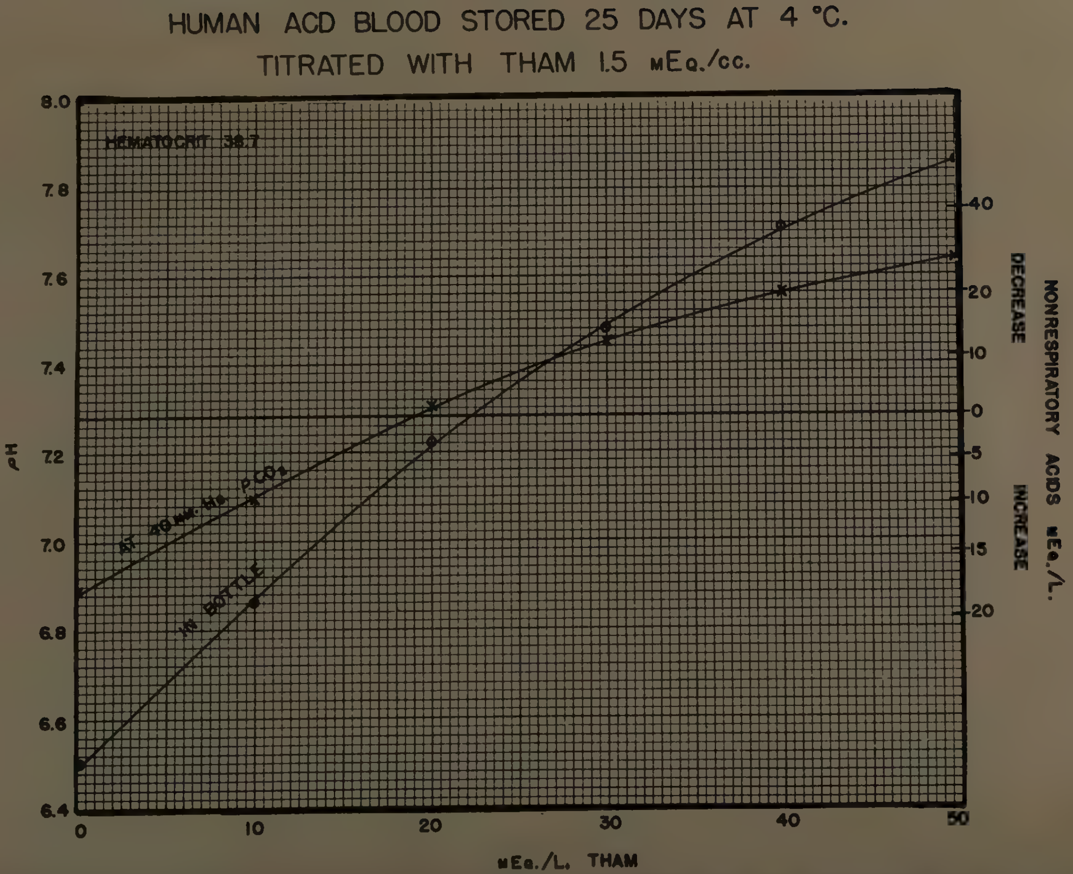
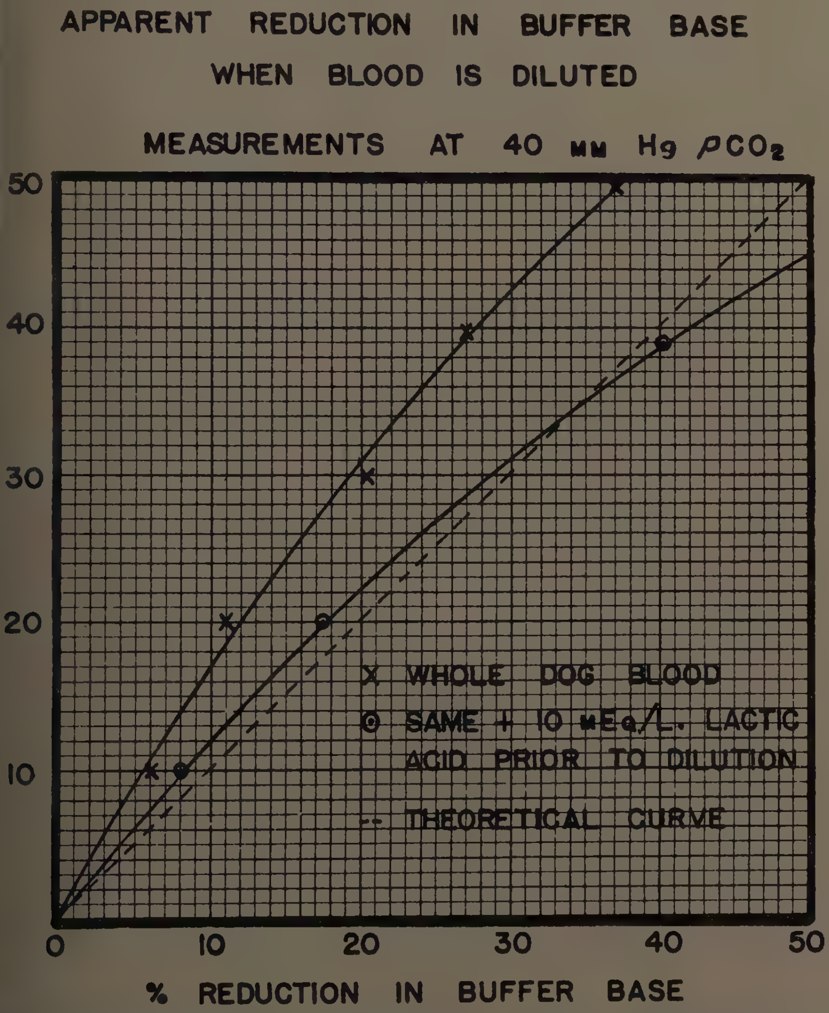


FIGURE 3. When the mixed acidosis of stored ACD blood is treated with Tris (THAM), the pH is raised even though the CO_2 is not allowed to escape. Thus this material may be used for respiratory acidosis, nonrespiratory acidosis, and mixed acidosis. When the pCO_2 is high (low pH), the action of the Tris is enhanced by keeping the blood at 40 mm. Hg, but when the pCO_2 is low (high pH), the addition of CO_2 decreases the

ent is on total cardiopulmonary bypass on the membrane lung, acidosis
not be corrected by the administration of sodium bicarbonate. FIGURE 7
cates the correction of nonrespiratory acidosis by Tris, even on prolonged



GURE 4. The acidosis produced by dilution has been found to be somewhat less than
l be expected if the buffer base were proportionately reduced. When the buffer base
lready been reduced by the addition of lactic acid, however, the reduction in buffer base
nes almost precisely proportional. In fresh ACD blood the dilution acidosis is approxi-
y 0.14 *p*H units.

ss using the membrane lung. In both of these instances, antacids were
ired because of undue prolongation of the bypass due to ventricular
lation. In the first instance the fibrillation was never controlled, whereas
e second it was eventually controlled after the administration of Quinidine;
wing this, the heart performed well and the patient had an uneventful

TABLE 1

COMPONENTS IN THE ACIDOSIS OF ACD BLOOD PRESERVED 25 DAYS AT 4° C.

PCV (Hematocrit) 32, *p*H (37° C.) 6.51, pCO_2 (37° C.) 495 mm. Hg, total CO_2 33.5 mEq./l.

	PCV	Eucapnic *p*H*	Buffer base (BB) mEq./l.	Change in BB mEq./l.	Per cen of tota change
Initial blood	40	7.42	48.0	—	—
After 20% dilution	32	7.28	38.4	9.6	30
ACD added†	32	6.99	24.6	13.8	43
After desaturation	32	—	22.6	2.0	6
Incomplete metabolism in storage	32	6.80	15.9	6.7	21
				32.1	100

* *p*H at 40 mm. Hg pCO_2.

† Citric acid 880 mg. $= \frac{880 \times 3}{192}$ mEq. = 13.8 mEq./l.
(molecular weight = 192, valence = 3)

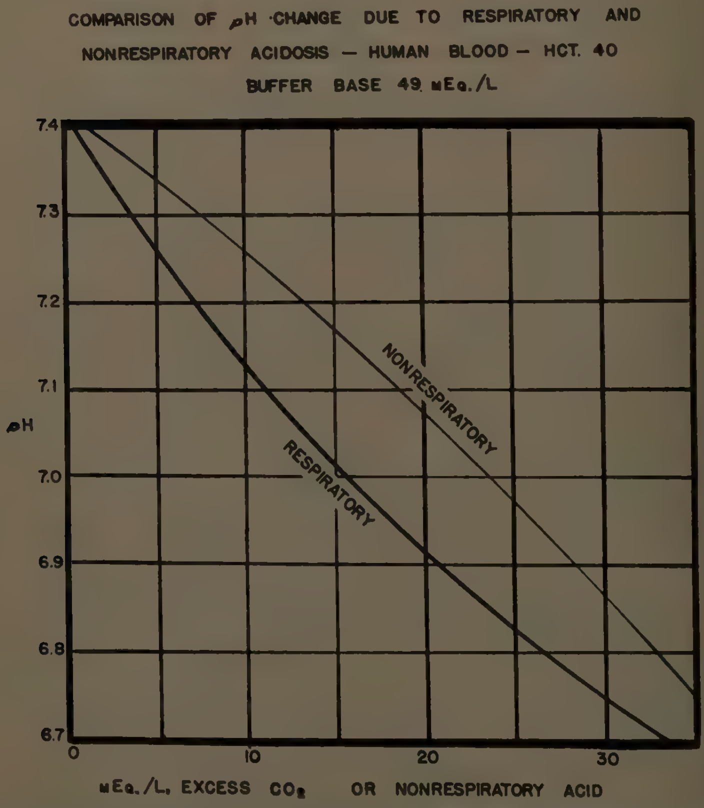
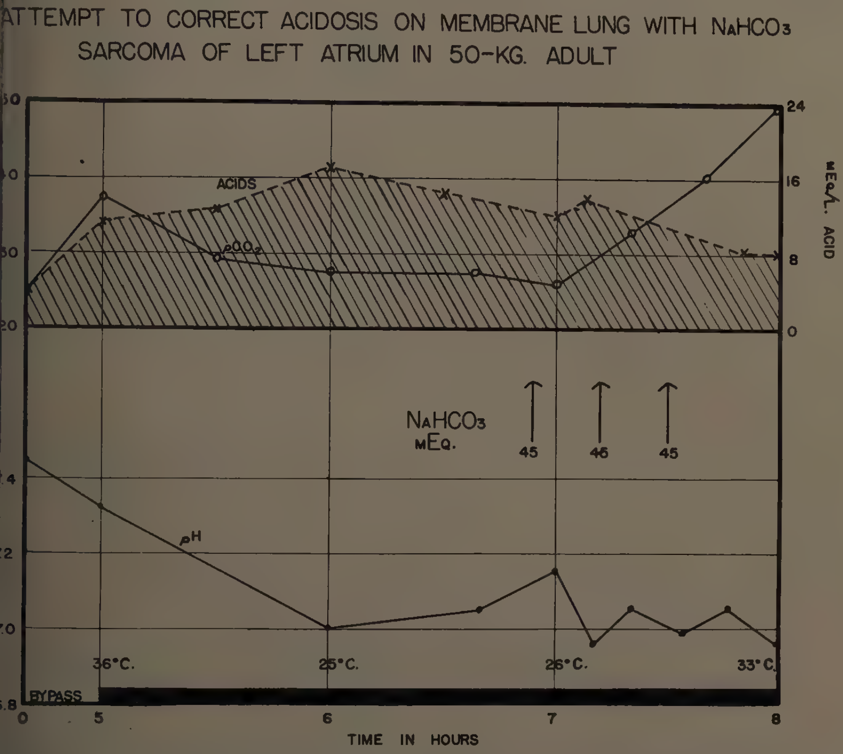


FIGURE 5. The reduction of *p*H by nonrespiratory and respiratory acidosis is illustra The nonrespiratory acid line is at a pCO_2 of 40 mm. Hg. The respiratory acidosis line is constant buffer base of 49 mEq./l. This chart is useful in estimating the initial dosag Tris to be used in combatting respiratory or nonrespiratory acidosis (see text).

very. FIGURE 8 represents a more typical type of problem where Tris is rly indicated. Total circulatory arrest was utilized in deep hypothermia ermit repair of an infundibular stenosis and interventricular septal defect child with four venae cavae. There was a moderately severe nonrespira- acidosis after the arrest with a pH of approximately 7.14. This improved ntly on partial bypass during the rewarming phase and then showed a her tendency to fall despite the maintenance of a pCO_2 below 30 mm. Hg.



IGURE 6. In attempting to remove a sarcoma of the left atrium on cardiopulmonary by- under hypothermia, ventricular fibrillation occurred and could not be reverted, necessi- g prolongation of the bypass. A serious acidosis developed and because the patient on a membrane lung, which has a limited ability to eliminate carbon dioxide, the adminis- on of sodium bicarbonate raised the pCO_2 and failed to correct the acidosis.

pattern indicated circulatory decompensation and was accompanied by a in the venous pressure to a high of 25 cms. of saline. The acid-base abnor- ty was promptly and completely corrected following the administration of mEq. of Tris. There was improvement in the circulation as evidenced drop in venous pressure and a rise in arterial pressure. The patient made mpletely uneventful recovery.

Correction of Respiratory Acidosis

he correction of respiratory acidosis is seldom necessary during cardio- ionary bypass even when there is inadequate CO_2 elimination. Nahas

has shown that complete CO_2 retention leads to a declining pH and relativ rapid circulatory decompensation.[4] If the circulation is supported by adequate cardiopulmonary bypass, however, the circulatory depression d not take place and there is rapid recovery from the hypercapnic state on continuance of the bypass. Animals under such circumstances continue breathe and may not require any assistance to correct the respiratory abnorm ity; this situation is shown in FIGURES 9 and 10. An entirely similar situat is present if hypothermia is used in conjunction with CO_2 retention. colleagues and I have used 50 per cent carbon dioxide and prolon

CORRECTION OF ACIDOSIS ON MEMBRANE LUNG WITH THAM
CONGENITAL AORTIC STENOSIS IN 50-KG. ADULT

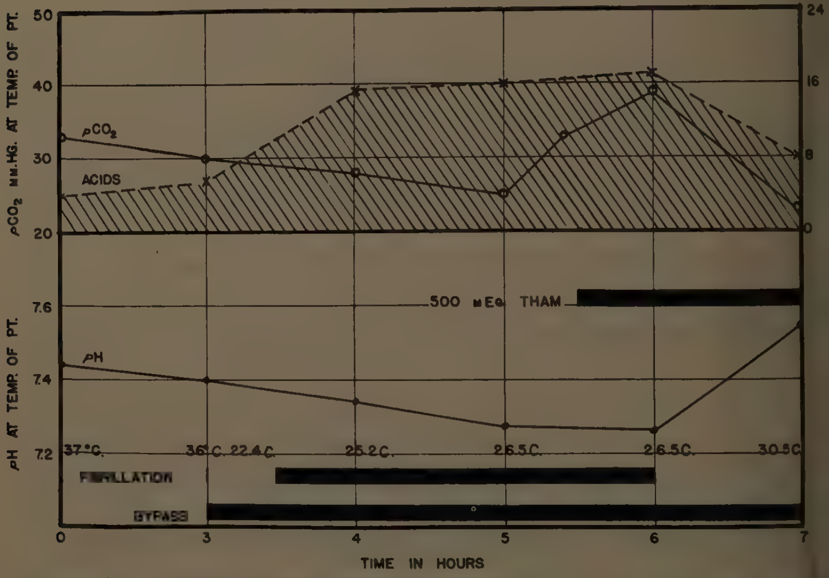


FIGURE 7. A moderate metabolic acidosis developed in a prolonged cardiopulmonary pass necessitated by the presence of ventricular fibrillation, which was difficult to re Administration of Tris (THAM) was effective in correcting the acidosis, even though membrane lung limits the elimination of carbon dioxide.

hypothermia to 8° C. without evidence of anything but transient respira depression. Because of this apparent lack of damage from a high pCO_2 du bypass and its rapid correction after blood flow through the lung and nor respiration are resumed, we consider it dangerous to give antacids for respira acidosis during bypass. In the postbypass period administration of anta for respiratory acidoxis should be limited probably to uncompensated sta secondary to pre-existing lung disease or lung damage, where mechar ventilation is not corrective.

Advantages and Disadvantages of Tris

Some advantages of Tris as an antacid during and following cardiopulmo bypass are summarized in TABLE 2, and disadvantages are shown in TABI

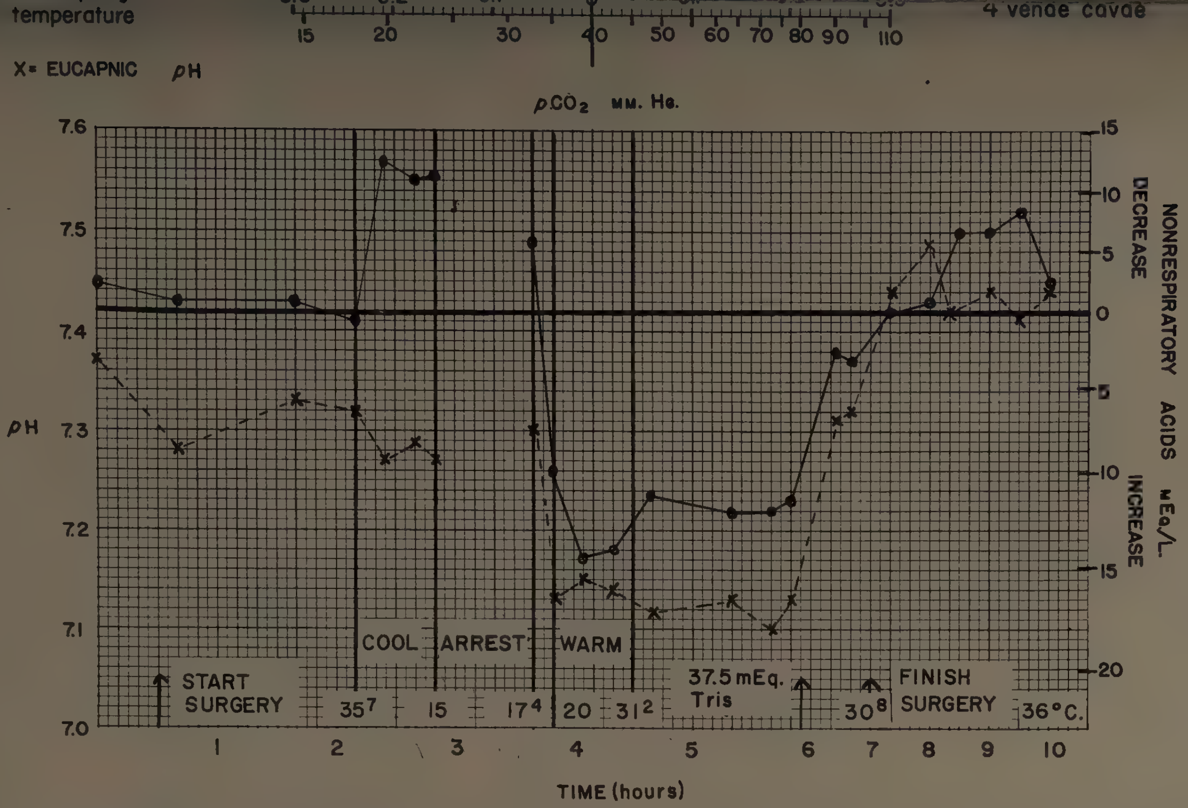


FIGURE 8. After completion of corrective surgery for a tetralogy of Fallot in deep hypothermia with total circulatory arrest, the eucapnic *p*H failed to rise, indicating the presence of a dangerous circulatory decompensation. This situation was accompanied by a rising venous pressure and a declining arterial pressure. Tris (37.5 mEq.) was administered on the basis of a nonrespiratory acidosis of 18 mEq./l. and an estimated blood volume of 2100 cc. The *p*H was relatively rapidly corrected to normal, and this was accompanied by the disappearance of the clinical signs of circulatory decompensation; the postoperative course was uneventful.

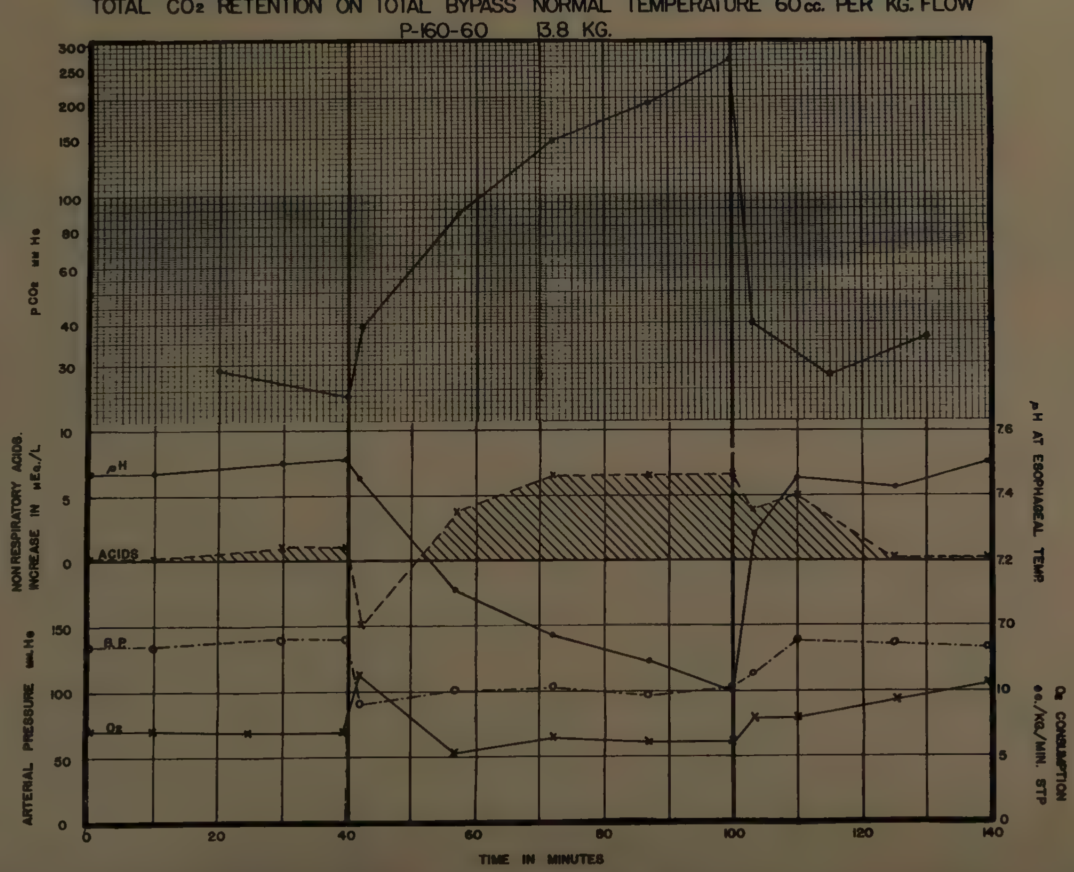


FIGURE 9. Closed-chest total cardiopulmonary bypass was instituted using a homologous lung in the extracorporeal circuit. The respirator to the homologous lung was arranged to deliver oxygen under positive pressure but without cycling, thus resulting in typical diffusion respiration and carbon dioxide retention. A pCO_2 rise to 270 mm. Hg was accompanied by a drop in pH to 6.8. The medium flow perfusion resulted in a slight decrease in

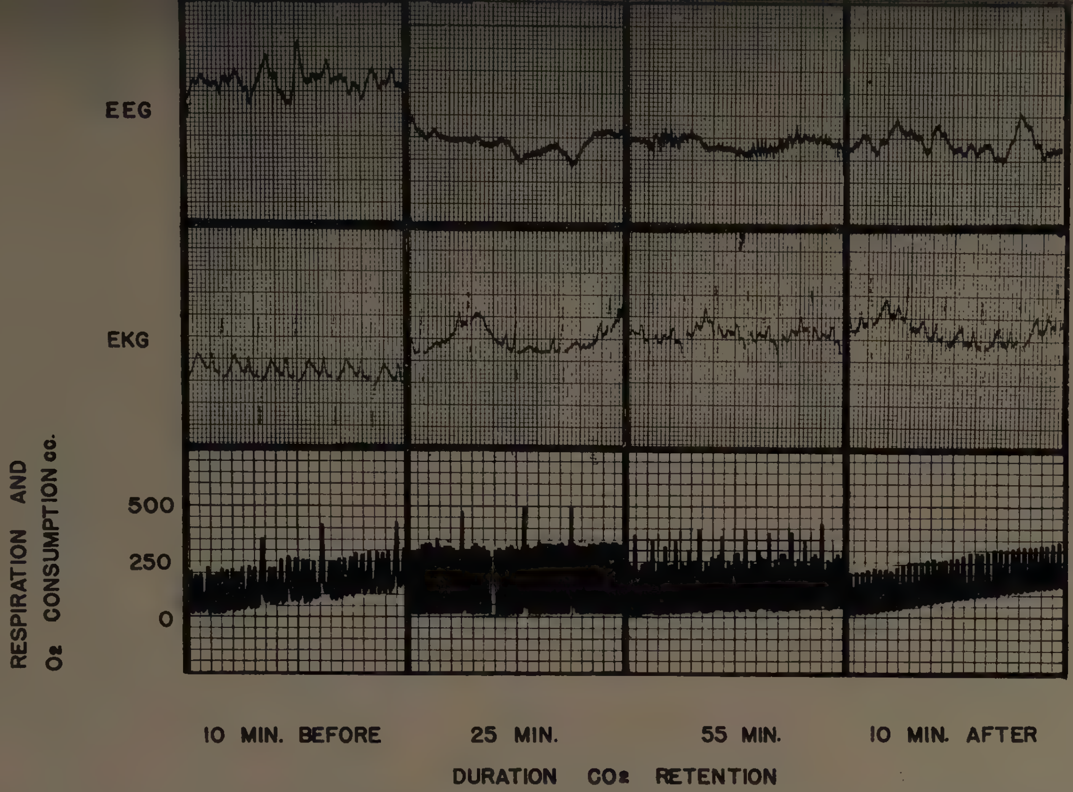


FIGURE 10. The EEG, EKG, respiratory activity, and oxygen consumption of the animal shown in FIGURE 9 are illustrated. The high pCO_2 did not cause abolition of the brain wave and permitted continuation of vigorous respiratory activity. All changes appeared quickly reversible.

Since moderate acidosis does not cause serious functional impairment, anta therapy is undesirable unless the arterial pH (at the temperature of the patie falls below at least 7.25 and shows evidence of a progressive decline. Th are a number of real drawbacks and dangers inherent in the administration antacids that should be clearly understood before these drugs are administe to patients during or immediately following cardiopulmonary bypass.

(1) Correction of the pH abnormality by an antacid effectively preve changes in pH from being utilized as a measure of respiratory and circulat normality. The situation is entirely analogous to the administration of pres drugs in the treatment of hemorrhagic shock. When this is done, it is extrem difficult to determine the adequacy of blood replacement and other more ba treatment measures.

TABLE 2
TRIS: ADVANTAGES

Effective in nonrespiratory, respiratory, or mixed acidosis.
May be able to correct intracellular acidosis.
Relatively nontoxic.
May be given without sodium.
Rapidly excreted in urine.
Enhances urinary carbon dioxide excretion.

TABLE 3
TRIS: DISADVANTAGES

May obscure cause of acidosis.
May have undesirable effects on blood sugar, serum potassium, and distribution of body water.
May depress respiratory center, aggravating hypoxia.
May cause undesirable urinary loss of water and electrolytes.

(2) If the patient has respiratory depression or an open chest, and requi mechanical ventilation, any prior treatment of respiratory acidosis or o treatment of nonrespiratory acidosis renders the patient subject to potenti dangerous alkalosis. Under such circumstances pH monitoring beco critically important. Even in the intact animal excessive administration Tris leads to an uncompensated nonrespiratory alkalosis. This is illustra in FIGURE 11; 300 mEq. of Tris neutralized with carbon dioxide to pH 7.0 given rapidly by the intravenous route. Despite the availability of la amounts of carbon dioxide, so that the animal did not have to retain metab carbon dioxide to neutralize the Tris, respiratory regulation maintained $p\mathrm{CO}_2$ at approximately 40 mm. Hg and permitted the pH to rise shar This is in line with the findings of others. The high pH at a $p\mathrm{CO}_2$ of 40 Hg is a reflection of the fact that 0.3 *M* Tris equilibrates above a pH of 8. body temperature when $p\mathrm{CO}_2$ is kept at 40 mm. Hg. This means that wh ever Tris is in excess, reduction of the $p\mathrm{CO}_2$ by hyperventilation may produ severe or even lethal alkalosis; this is clearly illustrated in FIGURE 12. A on total bypass at low flow was undertreated for total CO_2 retention with

ugh the pH was only partially corrected during the bypass, this was done expense of a moderate metabolic alkalosis. After the bypass, intermittent ve pressure ventilation was necessitated by respiratory depression. As as eliminated and perhaps intermediary organic acids further metabolized ntilatory reduction in pCO_2 produced a lethal alkalosis.

Dehydration and depletion of sodium and other electrolytes may result he diuretic action of the antacid.

In severe acidosis considerable quantities of fluid are necessary in order ninister the antacid, and hypervolemia may result.

Calculation of Antacid Dose

ause of the tendency for acidosis to be self-limited and overcorrection to ngerous, one should err on the side of undercorrection. The "addition" is of ACD blood is discussed above. In general, 10 mEq. of Tris is ctory per fresh unit, while 15 mEq. should be used when the blood is than 3 days old. This may be added directly to the blood if it is to be immediately; otherwise it should be given to the patient by a separate ion in a concentration of 0.3 mEq./cc.

treating a severe or progressive nonrespiratory acidosis it is useful to ate the dosage as though all of the increased acid is in the circulating volume. Theoretically, the nonrespiratory acids should be much more y distributed, but this has proved to be a good clinical way of measuring osage, and it avoids any possibility of dangerous overcorrection. The ximate blood volume in liters can be determined by assuming it to be /kg. The number of mEq./l. of Tris required is the same as the increase respiratory acids, the decrease in buffer base, or the decrease in "standard" onate.

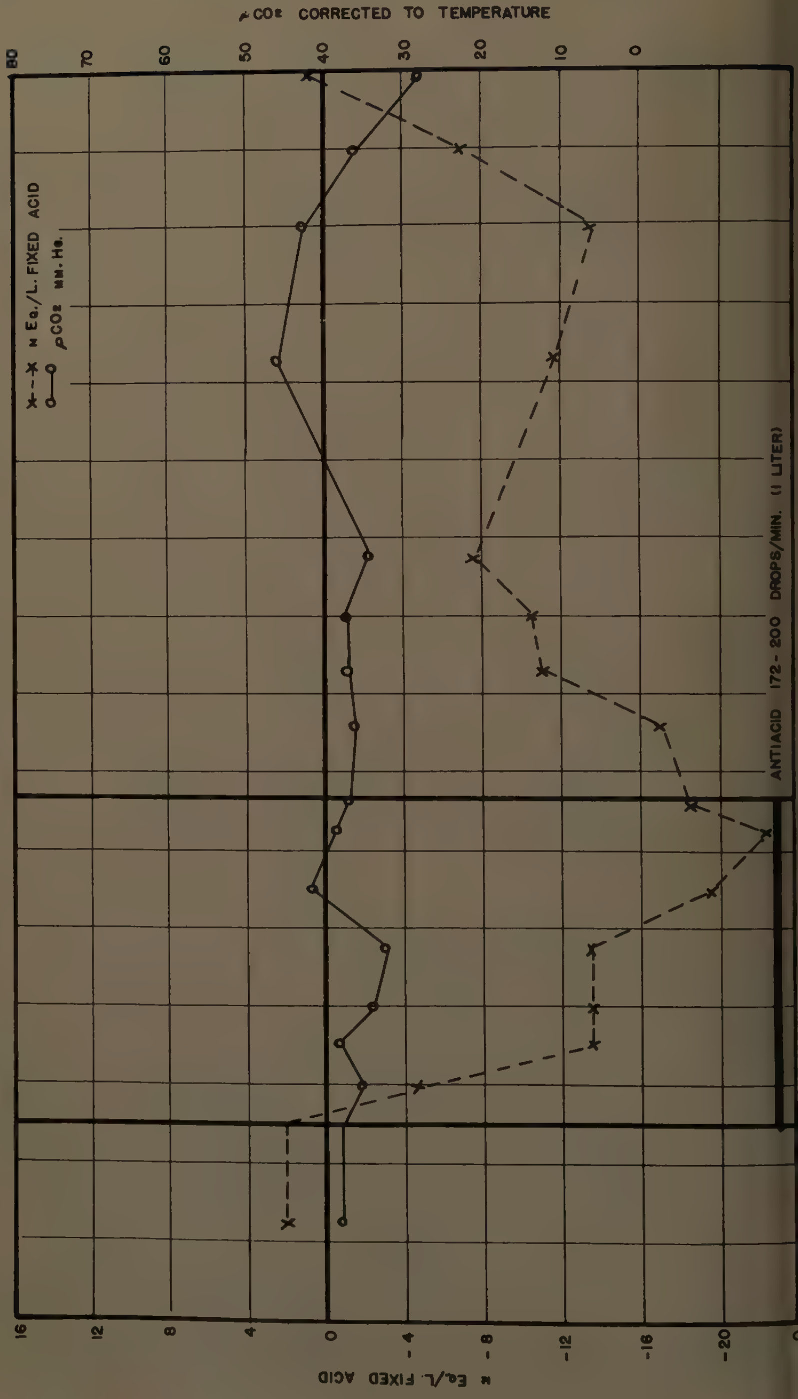
indicated earlier, correction of a respiratory acidosis with antacids is n necessary and probably undesirable. This condition should be cor- l by adequate ventilation with a mechanical respirator if necessary. ever it is desirable to correct a respiratory acidosis with Tris, a rough ate of the initial dose can be determined from FIGURE 5, but there is no tute for frequent pH determinations. The amount of Tris actually ed is determined by the difference between carbon dioxide production limination, and this is extremely difficult to estimate accurately under tions of partial retention because there is a variable increase in the carbon le retention, depending on the change in pCO_2 resulting from Tris ad- tration and the variation in respiratory activity secondary to effects of n the respiratory center. One should generally anticipate a respiratory ssion and possible hypoxia when Tris is given for respiratory acidosis ut the assistance of a mechanical respirator. All of these features make erally difficult to treat acute respiratory acidosis with Tris.

Summary

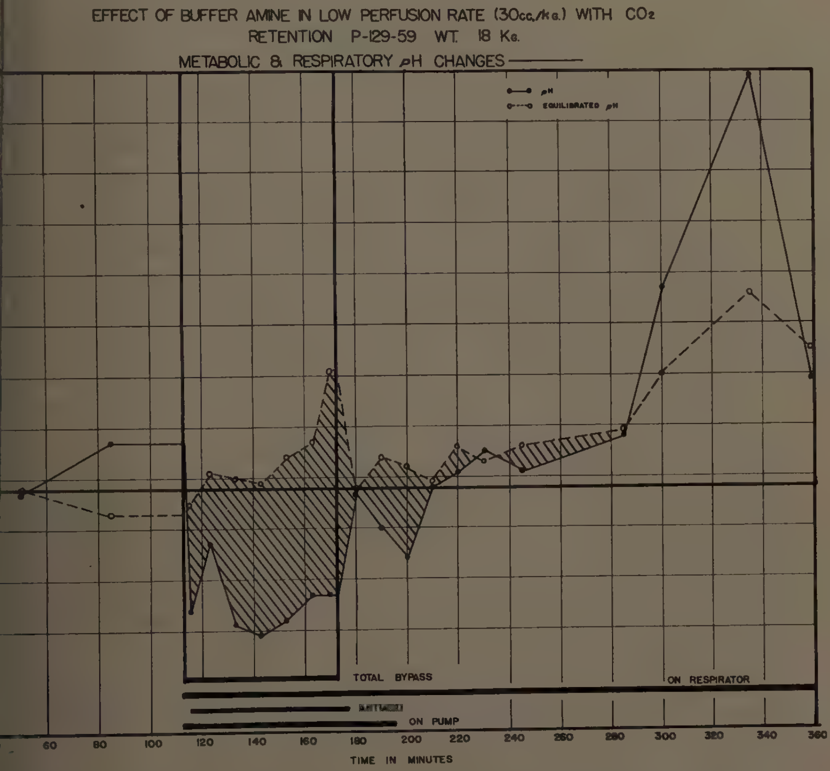
Serious acidosis should not occur in a well-conducted, routine cardio- nary bypass; therefore antacids should rarely be necessary. Generally ds should be withheld unless the pH falls progressively below 7.25 at the rature of the patient.

RESPIRATORY & METABOLIC ACID BASE CHANGES

EFFECT OF BUFFER AMINE (TRIS) CARBONATE (pH=7.0) IN NORMAL DOG P-144-59 WT. 14.3 KG.



Respiratory acidosis occurring during cardiopulmonary bypass is probably angerous and need not be treated. Respiratory acidosis occurring in the bypass period should be treated by adequate ventilation with a mechanical ator if necessary. Treatment of respiratory acidosis with antacids is to be difficult and dangerous.



GURE 12. A dog on closed-chest total cardiopulmonary bypass was perfused at a low of 30 cc./kg., while his homologous extracorporeal lung was carried on diffusion respira- permitting CO_2 retention. The respiratory acidosis was incompletely corrected by a lrip, and the combination of a low flow and severe carbon dioxide retention led to respira- lepression so that the dog had to be carried after the bypass on a respirator. Following tively stable period of nearly 2 hours, a severe mixed alkalosis and death ensued. The nce of large amounts of Tris interferes with normal acid-base control, so that the effects perventilation are markedly enhanced. Treatment of a self-limited respiratory acidosis fore should be avoided. When respiratory assistance is needed after the administration ge amounts of Tris, the *p*H should be carefully monitored.

GURE 11. The administration of 300 mEq. of Tris in the course of about 80 min. caused H to rise progressively to 7.73, indicating a severe "metabolic" alkalosis. Although the vas given with an ample amount of carbon dioxide so that the dog did not need to supply genous carbon dioxide to maintain a normal *p*H, there was apparently a preference for taining the pCO_2 at a normal level. The alkalosis continued for several hours, presuma- ntil most of the Tris had been eliminated via the kidneys. If Tris is given for a self- ed respiratory acidosis occurring during bypass, post-bypass correction of the CO_2 re- on will lead to nonrespiratory (metabolic) alkalosis. Reproduced by permission of A. *Archives of Surgery.*

(3) The level of nonrespiratory acids is the best index of circulatory integri
or adequacy of perfusion. Treatment of the acidosis with Tris may invalida
the usefulness of this index. In situations where there is progressive circu
tory decompensation, however, correction of the acidosis with Tris may rever
the decompensation.

(4) Sodium bicarbonate may be used for the treatment of nonrespirato
acidosis as long as respiratory function is intact. Tris, on the other han
may also be used in mixed acidosis where there is some difficulty in eliminati
of carbon dioxide. Tris may also be given without the addition of sodium, a
this on occasion may be very desirable.

(5) The acidosis of ACD blood is a composite state due to dilution, additi
of citric acid, accumulation of nonrespiratory acids, desaturation, and accum
lation of carbon dioxide. In fresh blood only the acidosis due to citric ac
need be corrected. In old blood the small additional nonrespiratory acido
should also be corrected.

(6) Methods of quantitating and treating acidosis occurring during a
after cardiopulmonary bypass are described and various illustrative cases a
presented.

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E USE OF 2-AMINO-2-HYDROXYMETHYL-1,3-PROPANEDIOL IN SEVERE CARBON DIOXIDE INTOXICATION*

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e neurological and circulatory manifestations of carbon dioxide intoxica- are now well recognized.[1,2] Clinically, the syndrome usually develops in nts with chronic lung disease when ventilation is further impaired by chial obstruction, pneumonia, lung collapse, heart failure, or respiratory ssion. If this condition is not recognized and treated effectively, the nt progresses into coma, circulatory failure, and death. Maintenance of ar airway and mechanical assistance to respiration are important for the ction of respiratory acidosis and its neurological and circulatory mani- tions.[2] Moreover, control of carbon dioxide accumulation in this way s time for specific measures to correct the factors acutely impairing ven- on. In spite of vigorous treatment, a patient may die if he is seen too or does not respond to adequate therapy. The use of buffer solutions, as sodium bicarbonate and sodium lactate, in this situation has not been uraging.[1,2]

ecently Nahas demonstrated that the organic buffer 2-amino-2-hydroxy- yl-1,3-propanediol (THAM†) is capable of preventing severe respiratory osis in dogs maintained with apneic oxygenation.[3] Moreover, animals ted with the buffer recovered rapidly when mechanical respiration was ided. These results suggested that THAM may be useful in the manage- t of severe carbon dioxide intoxication. Initially it was used in 3 patients, whom were maintained on mechanical respirators.[4] The results were ciently encouraging to prompt further evaluation of this agent in the ther- of carbon dioxide intoxication and respiratory acidosis. It is the purpose is report to describe our experience with the use of the organic buffer M in patients with severe carbon dioxide intoxication, in individuals with nic lung disease and hypercapnia, and in normal subjects.

MATERIAL AND METHODS

servations were made on five patients with severe carbon dioxide intoxi- n and on four with moderate hypercapnia and respiratory acidosis with- overt neurological or circulatory manifestations. Our experience with e of these patients has been previously reported.[4] The effects of THAM studied also in six healthy male medical students and physicians.

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THAM, as a solution of 0.33 *M* in 0.2 per cent NaCl, was administered i travenously to the patients at the rate of 300 to 500 ml./hour. The sa solution was used in the studies of normal subjects with infusion rates varyi from 300 to 900 ml./hour. Blood and urine samples were obtained and mea urements of ventilation were made before, during, and after the infusion. A terial blood oxygen saturation, *p*H, and carbon dioxide tension were determin in all studies. Serum electrolytes and urine volume, *p*H, sodium, and pota sium were determined in some patients with severe carbon dioxide intoxicatio Observations of minute ventilation were made with some patients and wi the normal subjects. The effects of breathing low oxygen (7.5 per cent oxyg and 92.5 per cent nitrogen) and high carbon dioxide (5 per cent carbon dioxi in air) gas mixtures were studied in the normal subjects, with measuremen of blood gases and minute ventilation before and during THAM infusion.

Blood oxygen saturation was determined by the photometric method Hickam and Frayser.[5] Blood carbon dioxide was determined by the meth of Peters and Van Slyke.[6] The *p*H of whole blood was measured by the Ca bridge model R *p*H meter, with measurements corrected to 37° C.[7] In so instances the *p*H was measured by the same type *p*H meter with the gla electrode enclosed in a 37° C. water bath. Plasma carbon dioxide conte was calculated from the blood carbon dioxide content, *p*H, and hemoglob using the line charts of Van Slyke and Sendroy.[8] Carbon dioxide tension w calculated from these values by means of the Henderson-Hasselbalch equatio using a *p*K of 6.11. Urine *p*H was measured by the Cambridge *p*H met Serum and urine sodium and potassium concentrations were measured on t standard Perkin-Elmer flame photometer. Serum and urine chloride conce trations were determined by the Volhard titration method. Minute venti tion was measured by collecting the expired air.

RESULTS

Patients with Carbon Dioxide Intoxication or Chronic Hypercapnia

THAM was given on one or more occasions to 9 patients with severe carb dioxide intoxication or with chronic hypercapnia and respiratory acidosis wi out overt neurological or circulatory manifestations of carbon dioxide retentio The results are summarized in the following case reports.

Case 1. A 51-year-old man was admitted to the hospital in April 1959 wi a protracted history of symptoms of chronic bronchitis, obstructive pulmona emphysema, and recurrent respiratory infections. Five years before adm sion he had developed symptoms and signs of congestive heart failure and fro that time had continued as a respiratory cripple with marked dyspnea at re He had frequent episodes of edema that required diuretic therapy. Seve days before admission, following an acute upper respiratory infection, he b came cyanotic and restless. On the day of admission he received a sedati intramuscularly and, following this, became unresponsive and cyanotic.

At the time of admission to the hospital he was in marked respiratory d tress, deeply cyanotic, comatose, and in shock. The blood pressure was 60 mm. Hg, and the pulse rate was 140/min. The anterior-posterior diame

e chest was increased and breath sounds were distant, with wheezes and
inspiratory and expiratory rales throughout both lung fields. The heart
enlarged and there was a loud ventricular gallop. The abdomen was ex-
ed and there was 2+ pitting edema of the pretibial and ankle areas. The
oglobin concentration was 15 gm. per cent and the WBC was 35,400/
mm. Urinalysis showed 1+ proteinuria and a pH of 5.1. Serum elec-
tes were within normal limits. Chest roentgenogram showed a grossly
rged heart and bilateral increase in bronchial vesicular markings.
tracheostomy was performed at admission and the patient was breathed
a Jefferson respirator using 100 per cent oxygen. The arterial oxygen
ration was 97 per cent; pH was 7.21; pCO_2 was 105 mm. Hg. The patient
ved antibiotics, a digitalis preparation, diuretics, levarterenol, and corti-
roids. The blood pressure improved, but arterial blood gas findings of
ratory acidosis remained stationary for several days.

TABLE 1

EFFECT OF THAM IN A PATIENT BREATHED BY A RESPIRATOR*

Date	Condition	Arterial O_2 sat. (%)	Arterial pH	Arterial pCO_2 (mm. Hg)
6-1959	One day after adm.	100	7.19	134
10-1959	Control, respirator	100	7.31	90
	THAM infusion	100	7.34	88
	After THAM	100	7.43	77
14-1959	Control, respirator	100	7.31	106
	THAM infusion	98	7.40	96
	After THAM	99	7.39	93
21-1959		93	7.46	49

ase 1.

ter approximately 7 days with continued intensive therapy, the patient
ined semicomatose and infusions of THAM were administered intrave-
ly for 1 hour on 3 different days. On each occasion, the arterial pH in-
sed by 0.1 and the pCO_2 decreased by 13 mm. Hg (TABLE 1). During
nfusions of THAM the patient was breathed with a mechanical respirator.
narked change was noted in the patient's condition during the 1-hour in-
n except that he appeared more responsive. Except for an increase in
n potassium of 1 mEq./l., no changes were noted in serum electrolytes.
urine volume doubled on each occasion, for example, 80 ml./hour to 200
hour in one study. Sodium excretion was 10 mEq./hour before and 9
./hour after THAM infusion in this example, while potassium excretion
ased from 5 mEq./hour to 9.2 mEq./hour. One day later the patient
ed clearing of his sensorium and his respiration became less labored. He
weaned from the respirator and was ambulated several days later. He
discharged from the hospital with an arterial oxygen saturation of 93 per
pH of 7.46, and pCO_2 of 49 mm. Hg.

se 2. A 61-year-old man was admitted to the hospital in April 1959 with
ymptoms and signs of acute and severe cardiorespiratory failure. He
had respiratory distress related to chronic bronchitis and obstructive em-

physema for approximately 8 years. More recently he had had symptoms congestive heart failure.

At the time of admission, the patient was obese, cyanotic, and stuporo His blood pressure was 140/90 mm. Hg. The chest was emphysematous w inspiratory and expiratory rales. The liver was enlarged and there was pitti edema of the lower extremities. Hematocrit was 52 per cent and the wh blood count was 7,200/cu. mm. Serum electrolytes were within normal limi Chest roentgenogram showed pulmonary fibrosis and emphysema and heart shadow was enlarged.

The patient was started on antibiotics and intensive anticongestive thera His arterial oxygen saturation was 72 per cent and the *p*H was 7.11. Trac ostomy was performed and, at that time, the arterial blood *p*H was 6.97 a $p\mathrm{CO}_2$ was 120 mm. Hg. Following this the patient was breathed with a po tive-negative resuscitator and, after three hours, the *p*H was 7.13. THA was administered during a one-hour period, with the results shown in TABLE The patient appeared more alert and no significant changes in serum elect lytes or urine volume and electrolytes were noted. With continued assista to ventilation, the patient's condition improved, he became oriented, and respiratory distress was less pronounced. Five days later he was afebrile breathing spontaneously. Three weeks later he was discharged from the h pital in satisfactory condition.

Table 2

Effect of THAM Used in Combination with a Respirator*

Date	Condition	Arterial O_2 sat. (%)	Arterial *p*H	Arterial *p*C (mm. Hg
4-25-1959	Tracheostomy, respirator		6.97	121
4-25-1959	Control, respirator	91	7.14	86
	THAM infusion	91	7.30	76
	After THAM	91	7.16	86

* Case 2.

Case 3. A 48-year-old man was admitted to the emergency room in A 1959. He had had asthma of 2 years' duration but this had become m more severe during the 2 weeks before admission. In the emergency room patient was cyanotic and comatose. Blood pressure was 150/30 mm. Hg pulse rate was 130/min. Breath sounds in the chest were decreased bi erally and there was a loud ventricular gallop over the precordium. Arte blood showed an oxygen saturation of 67 per cent, *p*H of 6.96, and pCO 133 mm. Hg.

Bronchoscopy was done and tracheostomy was performed. Infusion THAM was started while the patient was begun on mechanical respirat Within 20 min. after the tracheostomy and the infusion of THAM the pat died. An autopsy revealed mucous plugs in the bronchi and bronchio bronchitis, emphysema, and atelectasis. No other remarkable changes observed in the other organs of the body that might have been related to THAM infusion.

ise 4. A 49-year-old man was admitted to the hospital in August 1960
a history of cough, wheezing, and shortness of breath for many years.
hin the 5 weeks before admission, he had become progressively more short
reath and developed abdominal swelling and ankle edema. He had re-
ed therapy for congestive heart failure but had not responded.

the time of admission to the hospital, the patient was cyanotic and in
ous respiratory distress. Blood pressure was 118/65 mm. Hg and the
rate was 116/min. The anterior-posterior chest diameter was increased,
the breath sounds were distant with many rhonchi scattered throughout
lung fields. The liver was enlarged and there was evidence of abdominal
. There was 1+ pitting edema at the ankles. Hemoglobin was 15.2 gm.
ent, and the white count was 14,900/cu. mm. Serum electrolytes were
markable, except that the serum potassium was 5.1 mEq./l. Chest roent-
gram showed increased pulmonary vascularity, diffuse infiltrate through
ight lung field, and cardiac enlargement. Shortly after admission, arterial
d oxygen saturation was 83 per cent, pH was 7.24, and pCO_2 was 95 mm.

TABLE 3

EFFECT OF THAM IN PATIENT WITH UNASSISTED RESPIRATION*

Date	Condition	Arterial O_2 sat. (%)	Arterial pH	Arterial pCO_2 (mm. Hg)	Ventilation (l./min.)
30-1960	Control	73	7.30	82	11.0
	THAM infusion-40′	75	7.32	87	7.0
	THAM infusion-60′	69	7.34	88	
	After THAM	68	7.31	90	10.0

Case 4.

llowing admission the patient was started on antibiotics and corticos-
ds, and was continued on anticongestive therapy. In addition he re-
ed low-flow nasal oxygen with periodic use of intermittent positive-pressure
thing. The patient, however, continued to have severe respiratory dis-
and cyanosis when not receiving oxygen. In addition, he became gradu-
more confused, disoriented and, eventually, semistuporous. THAM in-
n was given, with the results shown in TABLE 3. No appreciable change
e patient's mental status was observed, and marked decreases in minute
ilation and oxygen saturation were noted. THAM infusion was discon-
ed and the patient received his previous treatment program. With a
ht loss of approximately 13 kg. he showed improvement and, at the time
scharge, the arterial oxygen saturation was 85 per cent, pH was 7.38, and
$_2$ was 44 mm. Hg.

se 5. A 63-year-old white man was admitted to the hospital in August
. He had a history of symptoms of chronic lung disease for approximately
ears. He had been previously treated for carbon dioxide narcosis with a
eostomy and Jefferson respirator with good response. Two days before
ssion he developed an acute upper respiratory infection, marked dyspnea,
yanosis.

At the time of admission to the hospital the patient was obese, cyanoti
obtunded, and in acute respiratory distress. The anterior-posterior diamet
of the chest was increased, and the breath sounds were distant. The liver w
enlarged and there was 2+ pitting edema of the ankles. The hemoglobin w
16.5 gm. per cent and the white blood count was 12,200/cu. mm. Serum ele
trolytes were within normal limits, except that the potassium was 5 mEq./
Chest roentgenogram showed fibrotic changes in the chest with emphysem
and cystic areas. Arterial blood oxygen saturation was 73 per cent, pH w
7.28, and $p\text{CO}_2$ was 91 mm. Hg.

A tracheostomy was done and the patient was breathed with an automat
intermittent positive pressure breathing device. Following 24 hours of th
management, which also included antibiotics and corticosteroids, the arteri
pH was 7.35 and the $p\text{CO}_2$ was 77 mm. Hg. During the next several da
however, the patient remained stationary and continued to have spontaneo
muscle twitching and confusion. He received an infusion of THAM during
1-hour period while receiving low-flow nasal oxygen without support by m
chanical respiration. The results are shown in TABLE 4. The arterial oxyg

TABLE 4
EFFECT OF THAM WITH RESPIRATION UNASSISTED*

Date	Condition	Arterial O_2 sat. (%)	Arterial pH	Arterial pCO (mm. Hg)
9-2-1960	Control	93	7.32	65
	THAM infusion	100	7.32	72
	After THAM	99	7.31	71

* Case 5.

saturation and pH did not change appreciably. The CO_2 content increase
however, and the $p\text{CO}_2$ rose by 7 mm. Hg. The infusion was discontinu
and the patient managed with low-flow oxygen, periodic use of intermitte
positive pressure breathing, corticosteroids, antibiotics, and diuretics. He i
proved through the course of the next 7 to 10 days and was discharged mod
ately well compensated.

Case 6. A 60-year-old white man was admitted to the hospital in July 19
He had had severe exertional dyspnea for 7 years. On previous admissio
to the hospital a diagnosis of pulmonary emphysema had been made, and t
patient demonstrated ventilatory insufficiency with arterial oxygen unsatu
tion and carbon dioxide retention. This admission was prompted by t
severe exertional dyspnea and respiratory distress.

On examination, the patient was depressed and cyanotic. The blood pr
sure was 160/80 mm. Hg. The anterior-posterior diameter of the chest w
increased, the diaphragm was low, and breath sounds were distant. The li
was not enlarged, and there was no peripheral edema. The hematocrit was
per cent. Serum electrolytes were within normal limits, except that the seru
potassium was 5.7 mEq./l.

Although the patient was oriented, he remained quite depressed and
not improve with bronchodilator medications, low-flow nasal oxygen, a
intermittent positive-pressure breathing. A one-hour infusion of THAM

with no appreciable change in the patient's mental state. The results od gas studies are shown in TABLE 5. The patient was gradually comted but remained incapacitated at the time of discharge.

e 7. A 58-year-old white man admitted to the hospital in August 1960 ad recurrent attacks of bronchitis and asthma for 37 years. Ten days admission the patient's respiratory distress became more acute in ason with a respiratory infection. On examination he was in moderate atory distress with cyanosis of the nailbeds and lips. Blood pressure 40/70 mm. Hg. The anterior-posterior diameter of the chest was ind and the diaphragm was low. There was 1+ pitting edema of the extremities. The hematocrit was 55 per cent and the white blood count 3,880/cu. mm. Serum electrolytes were normal, except that the potas was 5.7 mEq./l.

Table 5

Effect of THAM with Respiration Unassisted*

Date	Condition	Arterial O_2 sat. (%)	Arterial *p*H	Arterial pCO_2 (mm. Hg)
5-1959	Control	87	7.33	57
	THAM infusion	72	7.38	61

ase 6.

Table 6

Effect of THAM with Respiration Unassisted*

ate	Condition	Arterial O_2 sat. (%)	Arterial *p*H	Arterial pCO_2 (mm. Hg)	Ventilation (l./min.)
-1960	Control	54	7.30	73	5.3
	THAM infusion	32	7.29	82	5.1
	After THAM	47	7.30	76	7.1

ase 7.

ile in the hospital the patient was maintained on intermittent positiveure breathing, low-flow oxygen, and bronchodilator drugs. In addition ceived therapy for congestive heart failure. An infusion of THAM was with the results shown in TABLE 6. Minute ventilation was not measy altered when compared with the control but, with an increased rate, volume fell. In the postinfusion period minute ventilation was increased. arterial oxygen saturation decreased, and carbon dioxide retention was er during the THAM infusion. The patient noticed no particular change state.

se 8. A 69-year-old white man was admitted to the hospital in September because of a chronic cough and exertional dyspnea. The diagnosis of hogenic carcinoma had been made on a previous hospitalization in Feb 1959, and the patient had received X-ray therapy. On examination he dyspneic and cyanotic. The blood pressure was 130/80 mm. Hg, and ulse rate was 100/min. Chest movement was limited, and expiratory wheezes were heard throughout both lung fields. There was no hepatomeg or ankle edema.

The patient received antibiotics and bronchodilator drugs. In addit intermittent positive-pressure breathing was used. An infusion of TH was given; the effects on blood gases and ventilation are shown in TABL The minute ventilation decreased during the infusion, and the patient no no change in his state.

Case 9. A 31-year-old white man, admitted to the hospital in Septem 1960, had a history of recurrent lower respiratory infections. Exertic dyspnea had become gradually more severe and, 2 months before admiss ankle edema and abdominal distention were noted. On examination patient was in respiratory distress. The blood pressure was 95/60 mm. and the pulse rate was 120/min. The anterior-posterior diameter of the c was increased and the breath sounds were distant. Arterial oxygen saturat was 74 per cent, pH was 7.30, and pCO_2 was 70 mm. Hg.

TABLE 7
EFFECT OF THAM WITH RESPIRATION UNASSISTED*

Date	Condition	Arterial pH	Arterial pCO_2 (mm. Hg)	Ventilatio (l./min.)
9-20-1960	Control	7.45	44	11.0
	THAM infusion	7.48	42	7.8
	After THAM	7.43	54	8.0

* Case 8.

The patient was treated with bronchodilator drugs and intermittent p tive-pressure breathing. He was also given a digitalis preparation and uretics. THAM was infused and decreased his minute ventilation by 50 cent without an appreciable change in blood gases. After 4 weeks in the pital the patient was discharged markedly improved. He did not notice effects from the THAM administration.

Normal Subjects with Response to 5 per cent CO_2 and 7 per cent O_2 Breathi

The effect of THAM infusion on normal subjects was studied with part lar reference to response to 5 per cent CO_2 or 7 per cent O_2 breathing. results are summarized in TABLE 8. In 4 of the 6 subjects THAM infus decreased the minute ventilation by 1 to 2.6 l./min. In 3 the arterial oxy saturation decreased slightly, and pH and pCO_2 increased. Increase in mi ventilation in response to 5 per cent CO_2 breathing was less in 4 individ after THAM infusion but, when compared to the relative change in arte carbon dioxide tension, significant depression of the respiratory center was observed. In 1 of the 4 subjects less response to 7 per cent O_2 breathing observed after THAM infusion than before the infusion. In 3 of thes should be noted that the arterial oxygen saturation was lower and the p higher with 7 per cent O_2 breathing after THAM infusion, when compa to the control observation, although the ventilation was not significa different.

DISCUSSION

te hypercapnia with acidosis will produce toxic effects, but it is well nized that patients with chronic pulmonary disease tolerate considerable ion of the arterial carbon dioxide tension and compensate for the acidosis. ly with additional embarrassment of respiration and further carbon le retention, decompensation occurs and coma, shock, and death may . Successful treatment is dependent upon improvement of ventilation correction of respiratory acidosis. Occasionally this cannot be done

TABLE 8

EFFECT OF THAM IN NORMAL SUBJECTS AND ON RESPONSE TO 5 PER CENT CO_2 AND 7 PER CENT O_2 BREATHING

ubject	Inspired gas	Arterial O_2 sat. (%)		Arterial *p*H		Arterial pCO_2 (mm. Hg.)		Ventilation (l./min)	
		C*	T*	C	T	C	T	C	T
l. THAM sion	Room air 5% CO_2	96 96	97 97	7.38 7.30	7.43 7.30	44 53	42 61	12.0 19.9	13.3 11.9
l. THAM sion	Room air 5% CO_2	96 98	97 98	7.42 7.32	7.40 7.38	42 54	46 52	8.8 20.4	7.5 13.3
l. THAM sion	Room air 5% CO_2 7% O_2	96 97 73	94 98 70	7.38 7.32 7.44	7.38 7.32 7.46	43 53 38	50 59 41	8.8 17.5 17.3	7.8 16.8 18.5
l. THAM sion	Room air 5% CO_2 7% O_2	96 98 72	91 96 60	7.37 7.31 7.44	7.49 7.44 7.49	45 54 37	46 54 46	7.8 15.4 13.7	5.1 13.4 9.8
l. THAM sion	Room air 5% CO_2 7% O_2	97 97 68	97 97 62	7.37 7.32 7.44	7.47 7.38 7.47	49 57 41	48 65 50	6.7 15.5 11.6	4.4 12.8 12.4
l. THAM sion	Room air 5% CO_2 7% O_2	97 98 75	96 99 76	7 39 7.33 7.43	7.39 7.38 7.52	46 54 38	53 55 36	8.6 26.2 15.6	8.7 21.3 18.3

= control; T = THAM infusion.

ly enough to prevent death. In these circumstances a buffering agent t provide periods of compensation, allowing sufficient time for more spe- measures to correct the factors embarrassing ventilation. Sodium bi- nate and sodium lactate have not been particularly useful and have the vantage of requiring moderately large fluid infusion with high sodium nt.[1,2] Our earlier experience with THAM in critically ill patients with n dioxide narcosis suggested that this may be a good buffer to use for ensation of respiratory acidosis.[4] When THAM was infused during a d in which respiration was assisted with mechanical ventilator, the *p*H ased and the carbon dioxide tension fell. No alteration in arterial oxygen ation was noted. It was the clinical impression that at least one patient was somewhat more responsive after one hour of infusion. The fact tha dramatic change in mental state was not observed is not surprising, since has been demonstrated previously that even with prompt correction of respi tory acidosis improvement in mental state may lag by several hours.[2]

In patients with respiratory disease with compensated and uncompensa acidosis, but with unassisted respiration, THAM infusion resulted in an crease in arterial *p*H and carbon dioxide tension and a decrease in minute v tilation and arterial oxygen saturation. Other workers have reported a simi effect in patients with respiratory insufficiency when ventilation was unassis by a respirator.[9,10] This experience indicates that this buffering agent is tentially dangerous in patients with hypoxia, hypercapnia, and respirat acidosis if provisions are not made to correct oxygen unsaturation and as ventilation. It is our impression, however, that THAM is a useful adjunct the treatment of patients in whom intensive therapy, including the use mechanical respirators, is not successful or rapid enough in correcting acidosis.

Observations were made on the effects of THAM in normal subjects in attempt to elucidate the respiratory depression and arterial oxygen unsatu tion observed in the patients with unassisted respiration. Berman *et al.* ha reported a decrease in ventilation of 35 per cent, with an increase in arte *p*H and carbon dioxide tension, when THAM is infused into normal indiv uals.[11] In our study of normal subjects the response was variable, but in individuals the minute ventilation was decreased, although not always in as ciation with an increase in arterial *p*H or carbon dioxide tension. Brinkm has reported that THAM prevents the hyperventilation and acidosis associa with carbon dioxide inhalation in normal subjects.[12] In our studies the respo was slightly less with THAM, but in all cases except one (N. S.) ventilat did increase when 5 per cent carbon dioxide in air was breathed. The ex mechanism by which THAM depresses respiration, particularly in the patien is not clear. It appears unlikely that partial correction of an acid *p*H wo be effective in decreasing ventilation, since these patients are generally belie to be less responsive to changes in arterial carbon dioxide tension and *p*H th normal individuals. In some patients the fall in oxygen saturation af THAM infusion can be related to decreased ventilation, but it is of inter that in normal individuals the blood is more unsaturated following THA infusion and 7.5 per cent oxygen breathing than with 7.5 per cent oxyg breathing alone. Our data and that of others, however, suggests that THA is a respiratory depressant either by direct effect on the respiratory center through its effect on the *p*H.[9,12]

The manner in which THAM infusion decreases arterial carbon dioxide t sion when ventilation is assisted is not clearly understood. In normal subje Berman *et al.* have reported an increase in urine volume and in the excret of sodium, potassium, and bicarbonate.[11] In the limited observations ma in our study of the patients with severe carbon dioxide intoxication, an crease in urine volume was observed without striking changes in serum or ur electrolytes.

SUMMARY

The organic buffer 2-amino-2-hydroxymethyl-1,3-propanediol (THAM) was
en to patients with severe carbon dioxide intoxication, individuals with
percapnia and mild respiratory acidosis without neurological or circulatory
nifestations, and normal subjects exposed to both 5 per cent CO_2 in air and
per cent O_2 and 92.5 per cent nitrogen gas mixtures. When ventilation
s assisted, the arterial *p*H increased and the carbon dioxide tension fell.
hough striking changes in mental state were not observed, it is believed
t THAM is a useful adjunct in the management of severe carbon dioxide
oxication when ventilation is assisted. In patients with hypercapnia and
d respiratory acidosis THAM decreased ventilation, increased arterial car-
dioxide tension, and decreased the oxygen saturation when ventilation was
assisted. This response indicates that the agent should not be used unless
visions are made to maintain the patient's respiration. THAM infusions
normal individuals had a variable response but, in general, tended to de-
ase minute ventilation. Significant depression of response to carbon dioxide
alation was not observed. Hypoxia in normal individuals given THAM
reased ventilation, but the arterial blood was more unsaturated when com-
red to the control situation.

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THE USE OF TRIS(HYDROXYMETHYL)AMINOMETHANE DURING SHORT PERIODS OF APNEIC OXYGENATION IN MAN*

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During apneic (diffusion) oxygenation (ADO), that is, complete bloo oxygenation in the absence of tidal exchange, there is no elimination of carbo dioxide from the alveolar space (Holmdahl[1]). All carbon dioxide produced i retained in the body, giving an almost linear rise in the $PaCO_2$ in dogs durin the first half hour. This condition thus offers a useful experimental situatio with a well-defined acid load in investigations of the physiological efficiency o an injected buffer. Nahas[2] found that intravenous administration of approximately stoichiometric amounts of tris(hydroxymethyl)aminomethane (Tris o THAM) would neutralize all carbon dioxide produced and keep *p*H constan during 1 hour of ADO in dogs. All his dogs tolerated, without any apparen ill effect, 60 ml. of 0.33 *M* THAM per kg., that is, 2.4 gm./kg. administere during 1 hour. The rises in blood and cerebrospinal fluid pressures, otherwis observed during ADO, did not occur when the respiratory acidosis was buffere with THAM. The urinary output, which rapidly falls to zero during AD with uncompensated respiratory acidosis, after 20 min. was still equal to th fluid input when *p*H was kept constant by THAM infusion.

The above results led us to investigate the possible clinical use of THAM i conditions of respiratory and metabolic acidosis. The present report deal with the use of this compound to buffer the respiratory acidosis during a shor period of ADO in connection with bronchoscopies.

The Use of Apneic Oxygenation in Bronchoscopy

Since 1953 bronchoscopies under general anesthesia in the University Hospital of Uppsala have been performed with neuromuscular block by succinylcholine. The use of ADO under this procedure has been discussed previousl by the author.[1,3] The patient breathes oxygen in a nonrebreathing system t denitrogenate the lung, after which an ultrashort acting barbiturate (Pentotha or Narkotal, 200 to 500 mg.) is administered to insure unconsciousness throughout the procedure. Succinylcholine (1 mg./kg./body weight) is then injecte and the bronchoscope introduced, when the patient is completely paralyze as it is essential that no outside air containing nitrogen be drawn into the airway by an inspiratory movement.[1] During the whole procedure, oxygen i administered in a continuous stream (4 to 5 l./min.) through a small side-tub opening at the distal end of the bronchoscope. The wall of the distal one thir of the bronchoscope is perforated to allow the entire airway to be continuousl filled with oxygen. Otherwise, when the bronchoscope is pushed down an eventually completely fills one of the bronchi, the rest of the airway is not fille with oxygen. This technique effects apneic oxygenation during periods whe the bronchoscope is open and controlled ventilation with pure oxygen throug side arm of the bronchoscope is impossible. To insure complete blood oxy-enation, it is essential to administer succinylcholine in a dose sufficiently high o cause complete respiratory arrest. This may seem paradoxical, but it is xplained by the fact that if a little air is inspired, nitrogen will gain access to e lungs, and the alveolar oxygen tension will fall rapidly unless complete turn of spontaneous respiration with sufficient alveolar ventilation has ocurred. The continuous insufflation of oxygen through the bronchoscope does

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* A preliminary report of this study was presented at a meeting of the Swedish Anesthesiological Society, December 1959, in Stockholm, Sweden.

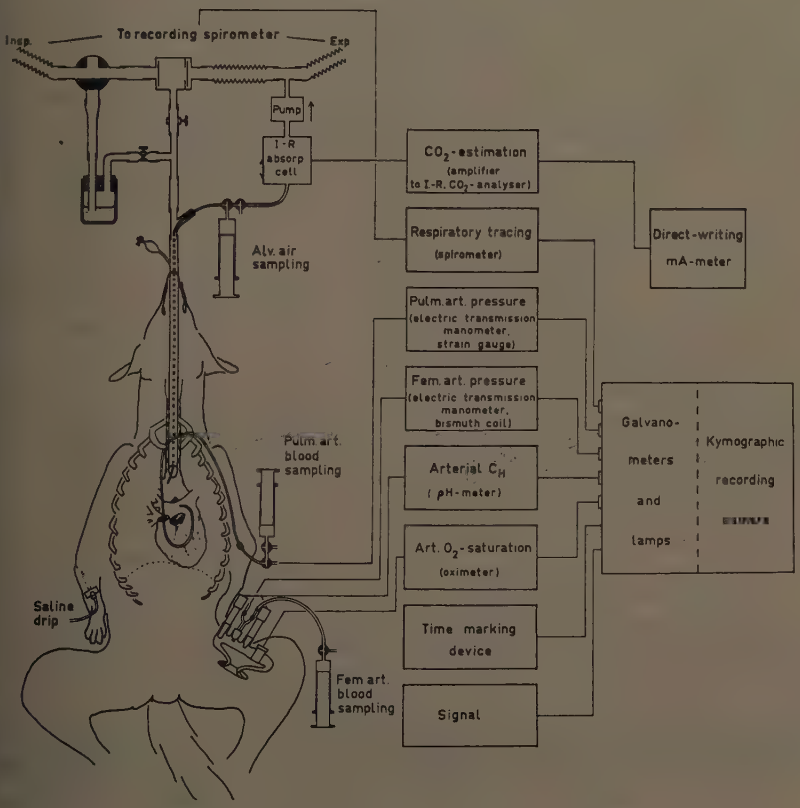
Insp.
To recording spirometer
Exp
Pump
I-R absorp cell
CO$_2$-estimation (amplifier to I-R. CO$_2$-analyser)
Direct-writing mA-meter
Respiratory tracing (spirometer)
Alv. air sampling
Pulm. art. pressure (electric transmission manometer, strain gauge)
Fem. art. pressure (electric transmission manometer, bismuth coil)
Galvano-meters and lamps
Kymographic recording
Pulm. art. blood sampling
Arterial C$_H$ (pH-meter)
Art. O$_2$-saturation (oximeter)
Saline drip
Time marking device
Fem. art. blood sampling
Signal

FIGURE 1. Schematic representation of methods used in experiments on dogs. (Reproduced by permission of *Acta Chirurgica Scandinavica*.[1]

ot produce any ventilation of the alveolar space. Its purpose is to maintain n atmosphere of pure oxygen in the airways essential for ADO. That no imination of carbon dioxide occurs during continuous intratracheal insufation and that the mechanism of oxygenation during continuous insufflation the same as that in ADO with the animal connected to an oxygen-filled pirometer has previously been demonstrated by the author[1] in dogs. In that udy the experimental setup allowed continuous registration of the oxygen satation and the *p*H of arterial blood flowing through a glass chamber inserted to the femoral artery (FIGURE 1), and the following results were obtained:

Figure 2 demonstrates that the decreases in pH during apneic periods ar identical when the denitrogenated animal is connected to a spirometer or whe insufflated with 5 l. of O_2 per min. through a small tube with the tip at th level of the bifurcation of the trachea. This experiment thus shows that con tinuous insufflation at the level of the bifurcation gives no elimination of CO during apnea.

Figure 3 again demonstrates the rapid fall in pH, from 7.4 to 7.1 during

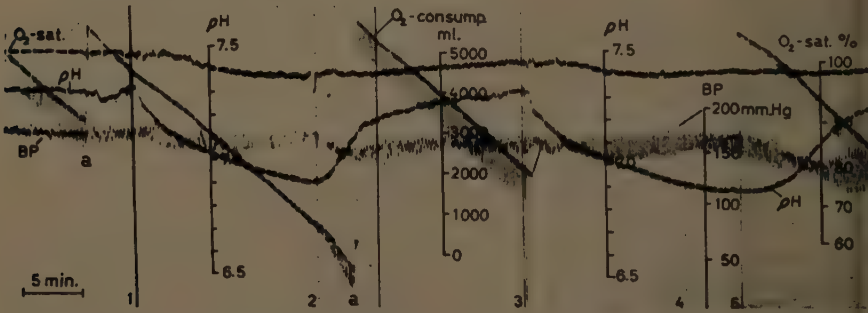

Figure 2. Curves showing similar decreases in pH in a dog (26.5 kg.) during ADO an during continuous intratracheal O_2 insufflation.)

Key: (1), succinylcholine, 0.5 mg./kg.; (2), spontaneous ventilation again sufficient t eliminate CO_2; (3), succinylcholine, 0.5 mg./kg., the tracheal tube then being disconnecte from the spirometer and O_2 insufflated continuously at the bifurcation of the trachea; (4), r appearance of small diaphragmatic movements; (5), tracheal tube reconnected to the refille spirometer; and (*a*), refilling of spirometer (owing to low period of the galvanometer, only mean blood pressure was obtained in this experiment).

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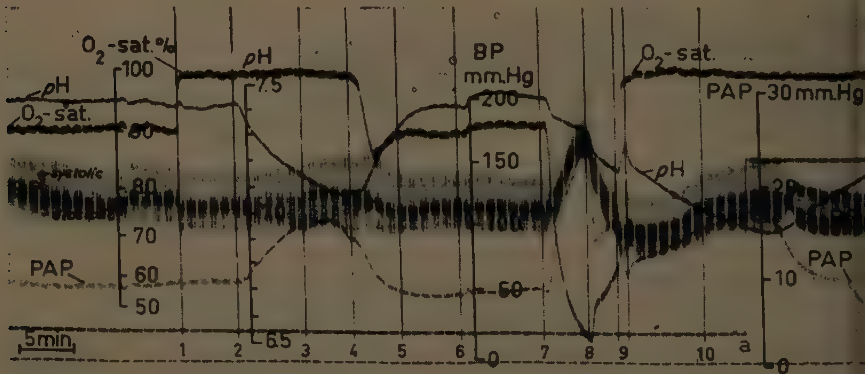

Figure 3. Maintenance of O_2 saturation during O_2 insufflation, and the rapid decrea during subsequent air insufflation. Dog No. 30, 20.2 kg.

Key: (1), continuous intratracheal O_2 insufflation started; (2), apnea induced with succin choline (0.35 mg./kg.); (3) returning diaphragmatic movements; (4), beginning of hyperpne causing air to be drawn into the lungs together with the insufflated O_2; (5), O_2 insufflati stopped; (6), insufflation of air; (7), succinylcholine (0.35 mg./kg.); (8) insufflation of O (9), between the two signals, intermittent insufflation of O_2; (10), returning diaphragma movements; and (*a*), refilling of the spirometer, which was not used in this experiment. PAP pulmonary arterial pressure.

Reproduced by permission of *Acta Chirurgica Scandinavica*.[1]

-min. apneic period, with insufflation of 20 l. of O_2 per min. at the level of furcation. It also shows immediate asphyxiation of the apneic dog, if air is bstituted for oxygen.

The Use of THAM in Bronchoscopies with a 6-Min. Apneic Period

Procedure. In order to investigate the buffering effect of THAM, an in- avenous infusion of a 0.33 *M* solution of the buffer in 0.2 per cent NaCl* as given during a 6-min. period of ADO in connection with bronchoscopies in 2 adult subjects. The changes in acid-base status were compared with those curring in 6 subjects not receiving THAM.

Ten of the subjects receiving THAM had lung tumors or small tuberculous vities and underwent bronchoscopy as a routine preoperative procedure. hey were all in good general condition without respiratory distress. Two bjects who will be dealt with separately were in bad condition with a ven- atory insufficiency prior to the bronchoscopy.

The infusion of THAM was started at a rate of 0.5 ml./kg./min. at the onset apnea when the bronchoscope was inserted. The 2 patients in respiratory stress were treated as follows: in addition to a 5-min. period of oxygen breath- g before the injection of Pentothal, they were ventilated with pure oxygen r 6 min. after the first injection of succinylcholine and before the broncho- opy was started.

Before the procedure, a polyethylene catheter was inserted percutaneously to the brachial or femoral artery by a modification of Seldinger's technique Iolmgren[4]). Arterial blood samples were drawn before and at the end of a min. apneic period, 5 min. after the apnea and 2 hours after the start of the ocedure. Following the apneic period, all patients were maintained on con- olled respiration until recovery of normal spontaneous respiration. Spon- neous breathing had returned in all patients when the first postapneic blood mples were taken.

The blood samples were immediately analyzed for pH and pCO_2, using an ectrometric technique described by Jørgensen and Astrup[5] with three pH terminations: one in the anaerobically collected sample and two at known rbon dioxide tensions. The actual HCO_3^- was calculated according to the enderson-Hasselbalch formula. The pO_2 was determined with a modified ark electrode. Brachial arterial blood pressure was measured by means of inflatable cuff and a mercury manometer.

Results. The changes in acid-base status occurring in 10 subjects infused th THAM during a 6-min. apneic period are shown in FIGURE 4 and compared th the changes in 6 subjects not infused with THAM.

During 6 min. of ADO with uncompensated total retention of carbon dioxide, CO_2 increased from 38 (range 32 to 44) mm. Hg to 66 (range 57 to 75) mm. with a fall in pH from 7.41 (range 7.36 to 7.46) to 7.24 (range 7.20 to 7.28) d a slight increase in actual bicarbonate from 24 (range 22 to 27) mEq./l. 28 (range 25 to 30) mEq./l. When 0.33 *M* THAM was infused in an aver- e amount of 0.53 (range 0.45 to 0.58) ml./kg. body weight/min., the arterial was kept almost constant during an identical apneic period with a rise of nost identical proportions in H_2CO_3 and HCO_3^- in all cases. The $PaCO_2$

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* Supplied by Pharmacia Ltd., Uppsala, Sweden.

rose from 37 (range 30 to 45) mm. Hg to 43 (range 32 to 49) mm. Hg and t HCO_3^- from 24 (range 21 to 27) to 28 (range 25 to 30) mEq./l. In all subjec the arterial blood was completely saturated with oxygen throughout t apneic period.

Five min. of artificial ventilation brought the arterial *p*H back to norm value, 7.39 (range 7.35 to 7.45), by again reducing the $PaCO_2$ to 43 (range to 50) mm. Hg in the control group, and kept the $PaCO_2$ constant and th the arterial acid-base status unchanged in the group that had received THA

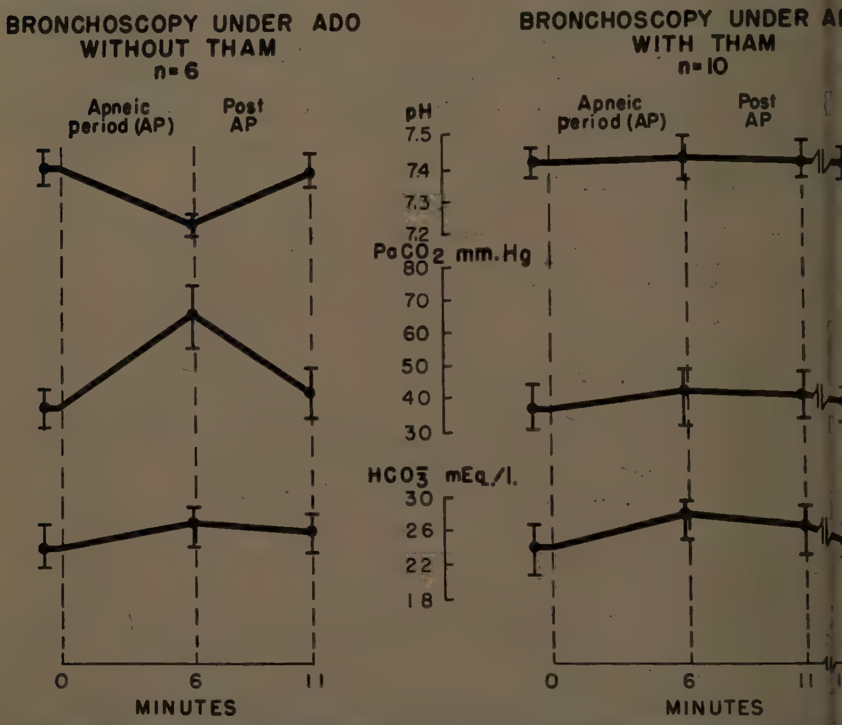
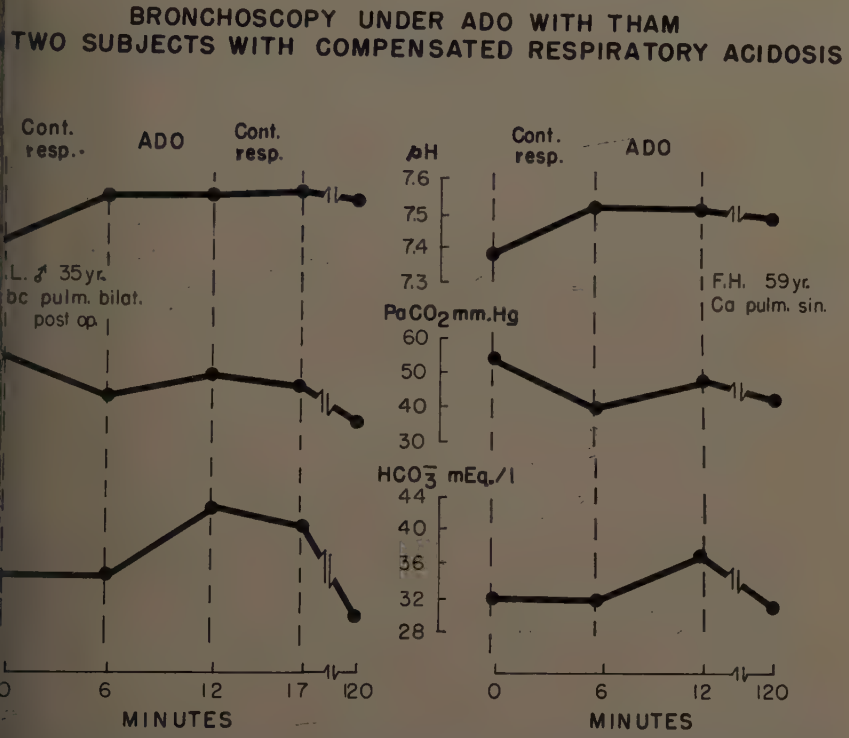


FIGURE 4. Acid-base changes during a 6-min. apneic period with or without infusio 0.33 *M* THAM (average amount 0.53 ml./kg./min.).

during the apneic period. In this latter group, the arterial HCO_3^- and Pa had returned to preapneic values after 2 hours.

Of the two patients in respiratory distress before the bronchoscopy, (E.L., FIGURE 5) had bilateral pulmonary tuberculosis and postoperative s nation of secretion after resection of the apicoposterior segment of the lung; the other (F.H., FIGURE 5) had a bronchial carcinoma with comp atelectasis of the left lung, and difficulties in clearing the airways from se tion. Both patients were slightly cyanotic when breathing air. Exposur pure oxygen for 5 min. relieved the cyanosis. Analysis of the arterial bl at that point in both subjects showed an almost fully compensated respira acidosis. The PaO_2 during oxygen breathing in E.L. was 109 mm. Hg, an

I. 162 mm. Hg, indicating shunting of nonoxygenated blood in the lungs. min. of controlled ventilation, before the bronchoscope was inserted, inased the PaO$_2$ slightly to 118 and 164 mm. Hg respectively, and caused a op in PaCO$_2$ to normal values with a marked alkaline shift in pH. During nin. of apneic oxygenation with infusion of THAM, the PaO$_2$ dropped only htly to 114 in E.L. and 152 mm. Hg in F.H., and the pH remained within .01 pH units, due to proportional increases in PaCO$_2$ and HCO$_3^-$. Re-



IGURE 5. Acid-base changes in two subjects during bronchoscopy with a 6-min. apneic od with infusion of 0.33 *M* THAM, 0.48 ml./kg./min., in subject E.L., and 0.54 ml./kg./ . in subject F.H.

val of secretions from the airways improved the respiratory status of both ients. They showed a slight spontaneous hyperventilation on breathing 2 hours after the procedure, with a PaO$_2$ of 87 mm. Hg and a PaCO$_2$ of 35 . Hg in patient E.L., and a PaO$_2$ of 80 and a PaCO$_2$ of 42 mm. Hg in pat F.H.

he systolic as well as the diastolic blood pressures, in all six subjects not eiving THAM, rose during the period of apneic oxygenation, the average ies immediately before and at the end of the apnea being 120/85 and 150/95 . Hg respectively. This consistent rise was absent in the series where the piratory acidosis was buffered with THAM. Here the blood pressure variins during the apneic period were much less pronounced, with slight in-

creases as well as decreases in pressure; the range being -10 to $+20$ mm.
for the systolic, and -5 to $+10$ mm. Hg for the diastolic.

In none of the subjects who received THAM were there any complaints
complications that could be referred to the period of apneic oxygenation or t
use of THAM. Two of the subjects, where THAM was not used, had hea
aches during the whole day after the bronchoscopy.

Discussion. During the 6-min. apneic period with continuous intratrache
insufflation of oxygen, after previous denitrogenation of the lungs, the aci
base changes were similar to those previously observed during apneic perio
in subjects connected to an oxygen-filled spirometer (Holmdahl[1]). It can
assumed, therefore, that all carbon dioxide produced during the apneic peri
was retained in the body. On the basis of stoichiometric reaction, 1.2 g
of THAM (equivalent weight 121.14) will buffer 224 ml. of carbon dioxide
standard temperature and pressure (STPD). If one assumes an average c
bon dioxide production of 200 ml./min. at STPD in adult man, an amount
around 1 gm. THAM, that is, 25 ml. of a 0.33 M solution, would be needed
min. to keep pH constant during apneic oxygenation. In the present ser
the average body weight was 64 kg. The infusion of 0.33 M THAM at
approximate rate of 0.5 ml./kg. body weight/min. was sufficient to neutral
the total carbon dioxide produced and maintain a constant pH. This amou
is slightly higher than could be expected from the above calculation in whi
however, rather uncertain figures for the amount of the produced and retain
carbon dioxide were used.

The findings of a less pronounced or absent blood pressure rise during
period of ADO, when the respiratory acidosis was buffered by THAM, is
accordance with earlier observations in dogs, made by Nahas.[2] The use
this amine buffer also eliminates the sudden and marked rise in cerebrospi
fluid pressure (CSFP) observed in dogs at the onset of a period of ADO (Naha
Also in man hypercapnia causes a rise in CSFP that can be eliminated by hyp
ventilation (Lundberg *et al.*[6]). Preliminary observations in our clinic, by
Gjessing, indicate that the use of THAM also in man eliminates a rise in CS
during apneic oxygenation. This may explain, in the subjects of our stu
the absence of headache, otherwise occasionally observed after periods of A
during bronchoscopy.

Preliminary results in our clinic indicate that 120 mg. THAM/kg. bo
weight (the approximate total amount of THAM given in this series), ove
period of 6 min. of apneic oxygenation, does not cause any hypoglycemia. T
may be in accordance with the observations reported by Tarail and Benne
who found only slight hypoglycemic activity with doses of 340 to 500 mg./
THAM given over 30 to 64 min. to three healthy human volunteers, wher
a dose of 1000 mg./kg. given during 1 hour to a fourth subject resulted i
marked transient hypoglycemia.

As there are no indications for using any longer periods of apneic oxygenat
in clinical practice, there may not be a place for the use of THAM of the t
outlined in this paper. In selected patients, where a blood pressure rise
intracranial pressure rise is undesirable, THAM may, however, be helpfu
counteracting respiratory acidosis, notably in procedures where adequ
ventilation cannot be given at all times.

Conclusion and summary. The intravenous infusion of 0.53 (0.45 to 0.58) nl./kg. body weight/min. of 0.33 *M* THAM keeps *p*H constant during a 6-min. eriod of apneic oxygenation with total retention of carbon dioxide in man. his is an amount only slightly higher than that expected on the basis of stoi-hiometric reaction between THAM and H_2CO_3.

The infusion of this amount of THAM was not followed by any toxic mani-estations.

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THE USE OF 2-AMINO-2-HYDROXYMETHYL-1,3-PROPANEDIOL IN THE MANAGEMENT OF RENAL ACIDOSIS*

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Nahas has noted the value of 2-amino-2-hydroxymethyl-1,3-propanediol (THAM) in the correction of respiratory acidosis induced by "apneic oxygenation" in the dog.[1] Other workers have shown that this agent is effective in the treatment of metabolic acidosis produced experimentally by the infusion of lactic acid to dogs with normal renal function.[2,3] Thus we became intereste in evaluating the possible use of this agent in the treatment of metabolic acidosis accompanying renal disease. This paper reports our experience with the use of THAM in the managements of patients with acidosis of renal origin.

Methods and Materials

Five adult patients with various forms of acute and chronic renal disease were selected for this investigation. All of these patients manifested a sever degree of acidosis and marked impairment of renal function as evidenced by a elevated blood urea nitrogen and low urinary output. Four patients receive 36 gm. and 1 patient was given 9 gm. of THAM intravenously dissolved in per cent dextrose and water. The period of infusion varied from 30 min. t 24 hours. Arterial blood pH and the total CO_2 content were determined before and immediately after the infusion of THAM. In most of these patient the blood pH and total CO_2 content were again determined after 24 hours Blood pH was determined anaerobically with a Beckman pH meter at roon temperature and corrected to 37°C. Total CO_2 content of blood was determined by the manometric method of Van Slyke and Neill.[4] Whenever possible the changes in serum electrolyte level were determined. However, becaus of the low urinary output no attempt was made to study the possible effects THAM on the urinary excretion of electrolyte.

Results

In order to elucidate the effect of administration of THAM on the clinica course and the acid-base balance, a brief clinical history of each of these patients will be presented separately. The possible beneficial effects and th observed complications of infusion of THAM will be indicated for each patien

Case 1, W.Z. (*4P 222*). This was the first Peter Bent Brigham Hospita (PBBH) admission of this 47-year-old white female (TABLE 1.) Twenty yea previously a diagnosis of glomerulonephritis had been made following an episo of illness characterized by edema, nausea, and vomiting. Seven years ag diastolic hypertension was noted for the first time. Throughout the pa everal years repeated urine examination had shown proteinuria. A week rior to admission the patient developed a severe sore throat that was adeuately treated with penicillin and tetracycline. The patient was admitted to nother hospital where her condition deteriorated with symptoms of nausea nd vomiting. On admission to Peter Bent Brigham Hospital, physical exmination revealed a B.P. of 200/120 mm. Hg. Examination of the optic undi revealed several flame-shaped hemorrhages. The heart was enlarged, nd there were many rales in both posterior lung bases. The laboratory exmination showed a hematocrit of 33 per cent. Urinalysis revealed 2+ proeinuria, 2 to 3 RBC, 3 to 5 WBC, and many granular casts. The patient as digitalized and started on parenteral protoveratrine for the treatment of ypertension. The urine output during the first 24 hours was 150 ml. Beause of severe metabolic acidosis the patient received 250 ml. of 0.3 *M* THAM uring a 30-min. period. Despite intensive therapy, the patient's condition ontinued to deteriorate and she died suddenly 24 hours after admission. IGURE 1 shows the initial plasma concentration of electrolyte and the effect of

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TABLE 1
W.Z. 4P222 47 W.F.

linical history:	Antecedent sore throat, nausea, and vomiting
hysical examination:	B.P. 200/120; Grade III retinopathy, cardiomegaly with congestive heart failure
aboratory data:	Hematocrit, 33; Urine, 2+ protein, 2–3 RBC, 3–5 WBC granular casts
rine output:	150 ml./24 hours
reatment:	250 ml. 0.3 *M* THAM 30 min.
iagnosis:	Acute glomerulonephritis
esult:	Death

HAM on blood *p*H and total CO_2 content. In this patient, no change in the erum concentration of sodium, potasium, or chloride was noted. There was, owever, a small but significant rise in blood *p*H and total CO_2 content.

Case 2, O.Z. (*4P392*). This was the first PBBH admission of this 55-year-ld white man because of progressive renal failure. As shown in TABLE 2, the atient apparently was in good health until about 2 months prior to admission hen he was admitted to another hospital because of weakness, fatigue, and xertional dyspnea. Hypertension and congestive heart failure were treated ith digitalis and chlorothiazide; because of progressive uremia he was transrred to PBBH. On admission his B.P. was 170/110 and his breathing was ussmaul in nature. The examination of the right optic fundus revealed any old exudates but no fresh hemorrhages. The heart was enlarged, and ere was moderate congestive heart failure. The laboratory examination owed a hematocrit of 33 per cent. Urinalysis showed a specific gravity of 012, 4+ protein; 5 to 10 WBC, and many granular casts. Because of modate acidosis, it was elected to infuse 1000 ml. of 0.3 *M* solution of THAM er a 4-hour period. FIGURE 2 shows the effect of infusion on the blood *p*H, tal CO_2 content, and the plasma concentration of electrolytes. Blood *p*H creased from 7.24 to 7.50, and the total CO_2 content increased from 7.0 to

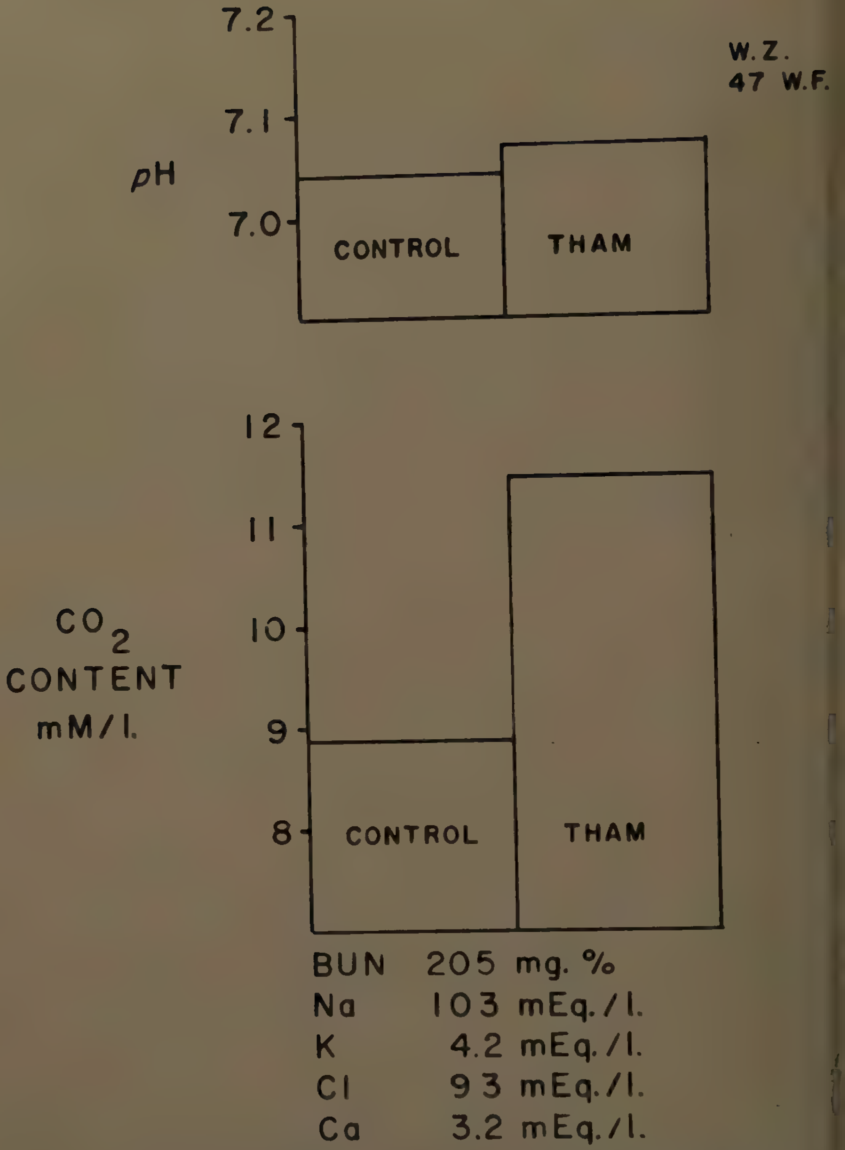


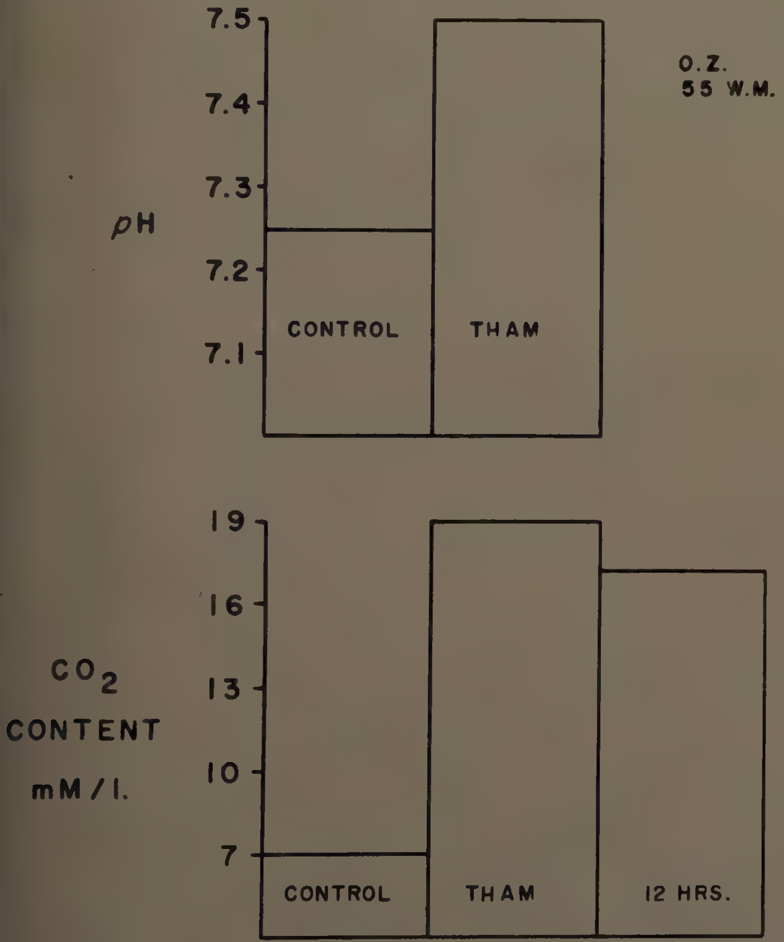
FIGURE 1. The result of infusion of THAM on the blood *p*H and bicarbonate level W.Z.

TABLE 2

O.Z. 4P392 55 W.M.

Clinical history:	Weakness, fatigue, exertional dyspnea
Physical examination:	B.P., 170/110; Kussmaul breathing; Grade III retinopath cardiomegaly with congestive heart failure
Laboratory data:	Hematocrit, 33; Urine, 4+ protein, 5–10 WBC, granular ca
Urine output:	800 ml./24 hours
Treatment:	1000 ml. 0.3 *M* THAM 4 hours
Diagnosis:	Chronic glomerulonephritis
Complication:	Hyperkalemia 18 hours after THAM
Result:	Death

0 mmoles/l. Eighteen hours following the administration of THAM the ient was found to be obtunded. Serum potassium level was 7.2 mEq./l. l EKG showed high and peaked T waves as well as widened QRS complex. e patient was treated effectively with hypertonic saline with immediate provement in the QRS complex. However, shortly thereafter he died ldenly before hemodialysis could be attempted.



	CONTROL	THAM	AFTER 12 HR.
BUN	220 mg.%	—	—
Na	141 mEq./L	134	134
Cl	104 mEq./L	98	98
K	6.7 mEq./L	7.1	7.2
Ca	4.2 mEq./L	4.3	—

FIGURE 2. The effect of infusion of THAM on blood and bicarbonate level of O.Z.

Case 3, W.J. (4P678). This patient was admitted to Peter Bent Brighar
Hospital for the first time from another hospital with a diagnosis of acut
glomerulonephritis. As shown in TABLE 3, he apparently was in good healt
until 4 weeks prior to admission when he developed a mild sore throat. Or
week later, bilateral flank pain and dark urine were noted. On admission h
appeared to be obtunded and had a Kussmaul breathing. The blood pressur
was 170/80, and there was evidence of congestive heart failure with cardic
megaly and atrial fibrillation at a rapid rate. The laboratory examinatio
showed a hematocrit of 24 per cent. Urinalysis revealed a specific gravity c
1.007, a trace of proteinurea, 4 to 6 RBC, 3 to 4 WBC, and occasional granula
casts. On the second hospital day, the patient received 2 l. of 0.15 *M* solutio
of THAM over a 24-hour period. As shown in FIGURE 3, the blood *p*H change
from 7.28 to 7.38, and the CO_2 content from 13.5 to 20.0 mmoles/l. The pa
tient continued to improve clinically, and the blood urea nitrogen (BUN
showed a steady fall until the 16th hospital day when the patient suddenl

TABLE 3
W.J. 4P678 59 W.M.

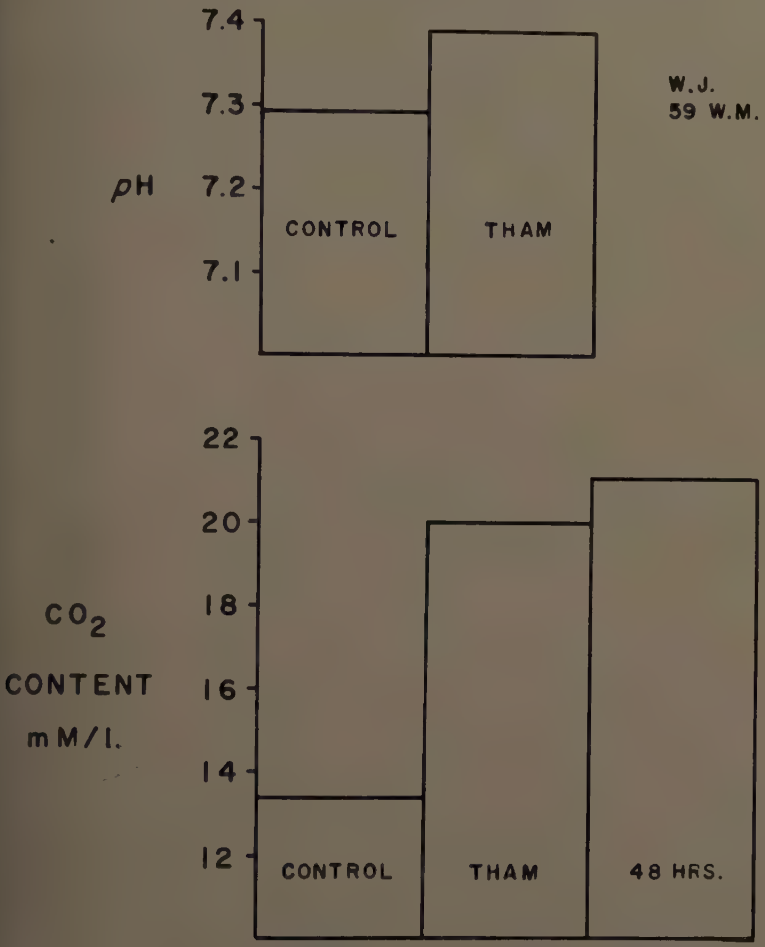
Clinical history:	Antecedent sore throat, flank pain, dark urine
Physical examination:	Obtunded; Kussmaul breathing; B.P. 170/80; cardiomegal and congestive heart failure; atrial fibrillation
Laboratory data:	Hematocrit, 24; Urine, sp. gr. 1.007, protein trace, 4–6 RB 3–4 WBC, and granular casts
Urine output:	1600–3010 ml./24 hours
Treatment:	2000 ml. 0.15 *M* THAM 24 hours
Diagnosis:	Acute glomerulonephritis
Complication:	Hemiparesis 14 days after THAM infusion; aspiration pne monia
Result:	Death

developed a right hemiparesis. On the 23rd hospital day, the patient vomite
and aspirated, resulting in cardiac arrest and death.

Case 4, G.S. (78886). As shown in TABLE 4, this was the second admissio
to the New England Deaconess Hospital, Boston, of this 31-year-old man with
14-year history of diabetes mellitus admitted because of progressive oliguri
weakness, lethargy, and diarrhea of 48-hours duration. On physical exam
nation the patient appeared acutely ill and was markedly dehydrated wit
pallor and Kussmaul breathing. Blood pressure was 160/80. Both opt
fundi showed flame-shaped hemorrhages with many hard exudates. Lung
were clear, but the heart was moderately enlarged. Laboratory findings co
sisted of a blood sugar of 975 mg. per cent and a hematocrit of 24 per cen
There was 1+ proteinuria, 2 to 3 WBC, and 8 to 10 hyaline casts in the urin
The urine output ranged from 100–200 ml./24 hour. The patient was treate
initially with 110 units of regular insulin and 250 ml. of 5 per cent saline. B
cause of persistent acidosis, 1 l. of 0.3 *M* solution of THAM was infused ov
the next 12 hours. FIGURE 4 depicts the results of the infusion of THAM
this patient. Blood *p*H increased from 6.92 to 7.36 and CO_2 content from 6
to 28.0-mmoles/l. Correction of acidosis resulted in an increase in the rate
urine flow from 100 to 700 ml./24 hours. The patient awakened from th

tunded state but lapsed back into coma and continued to remain oliguric. died on the 7th hospital day.

Case 5, R.S. (*4P393*). This was the first admission to Peter Bent Brigham spital of a 35-year-old housewife because of progressive anuria of 9 days'



	CONTROL	THAM	48 HR.
BUN	131 mg.%	155	170
Na	130 mEq./L	128	130
Cl	97 mEq./L	98	94
K	5.1 mEq./L	5.6	5.4
Ca	4.6 mEq./L	—	—

FIGURE 3. The changes in the blood *p*H and bicarbonate and serum electrolytes after administration of THAM to W.J.

TABLE 4

G.S. 78886 31 W.M.

Clinical history:	Diabetes mellitus 14 years duration. Progressive weakne fatigue, lethargy 48 hours
Physical examination:	B.P., 160/80; acutely ill, pale, dehydrated; fundi: bilate flame-shaped hemorrhage; cardiomegaly with modera congestive heart failure
Laboratory data:	Hematocrit, 24; Urine, 1+ protein, 2–3 WBC, 8–10 hyali casts
Urine output:	100–200 ml./24 hours
Treatment:	Hydration, saline, insulin, 1 l. 0.3 *M* THAM 12 hours
Diagnosis:	Intracapillary glomerulonephrosclerosis; diabetes mellitus
Complication:	Congestive heart failure
Result:	Death

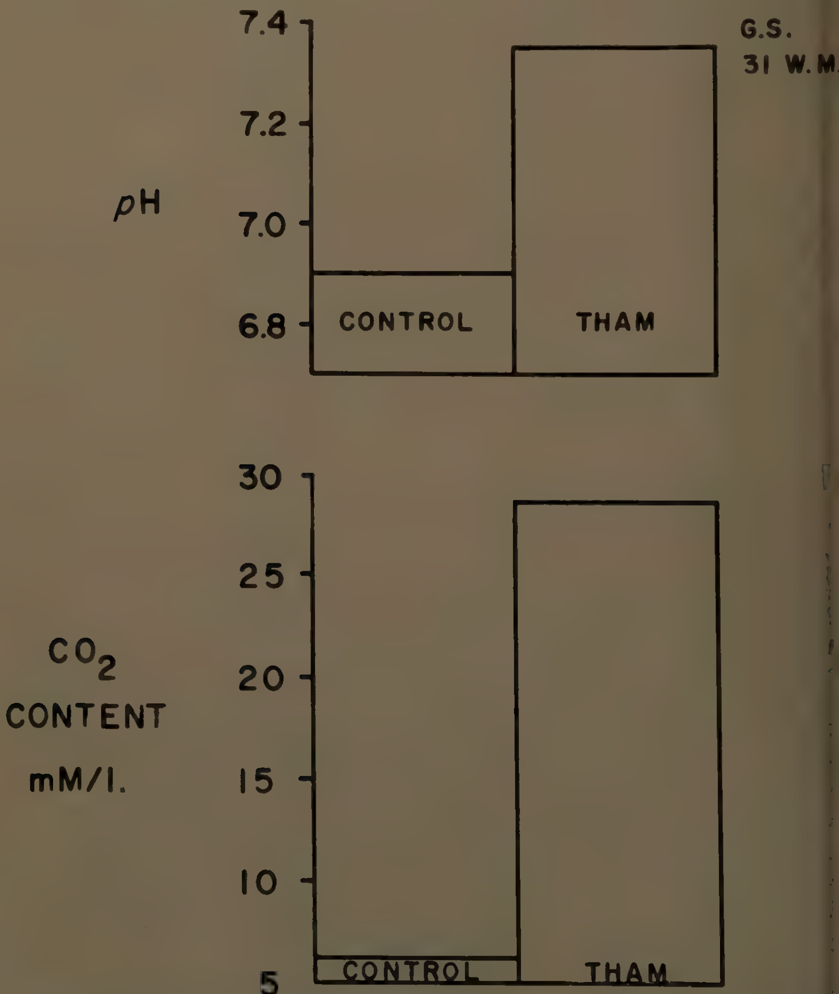


FIGURE 4. The effect of administration of THAM on the blood pH and bicarbon level of G.S.

ation. As shown in TABLE 5, the patient had developed generalized edema ing the first 4 pregnancies but had not been known to be hypertensive. ing the fourth month of her last pregnancy she developed hypertension. enteen days prior to admission a diagnosis of eclampsia was made on the is of hypertension, proteinuria, and convulsions that resulted in stillbirth subsequent anuria. On admission to the hospital her blood pressure was /80. The heart was not enlarged, and there was no evidence of congestive rt failure. The laboratory findings included a hematocrit of 37 per cent a urine specific gravity of 1.010, 2+ proteinuria, and occasional RBC and C in the urine. A lupus erythematosis (L.E.) cell preparation was ative, but the fluorescent antibody test was positive. The patient's 24-r urine output ranged from 1000 to 1200 ml. during the first 5 days of hoslization. Because of persistent acidosis, 500 ml. of a 0.6 M solution of AM was infused over a 4-hour period. FIGURE 5 depicts the resulting nges in the blood pH and total CO_2 content. It is evident that the blood changed from 7.22 to 7.44 and arterial CO_2 content from a control level of

TABLE 5
R.S. 4P393 35 W.F.

ical history:	Generalized edema during 4 previous pregnancies; hypertension, proteinuria, and edema during 4th month of present pregnancy; stillbirth and subsequent anuria
sical examination:	B.P., 145/80 no cardiomegaly or congestive heart failure
oratory data:	Hematocrit, 37; Urine sp. gr. 1010, protein 1+, occasional RBC and WBC; Flourescent antibody positive
e output:	1000–12,000 ml./24 hours initially
tment:	500 ml. 0.6 M THAM in 24 hours
gnosis:	Bilateral cortical necrosis
plication:	Hyperkalemia and oliguria 20 hours after THAM
ılt:	Eventual recovery—home.

to 15.0 mmoles/l. Shortly after the completion of the administration of AM, the patient became nauseated and vomited several times during the 12 hours. About 20 hours following the administration of THAM she ıme obtunded, and serum potassium level was found to be 7.7 mEq./l. with EKG alterations of hyperkalemia. Shortly afterward, the patient was yzed with a twin-coil Kolff artificial kidney with satisfactory lowering of serum potassium level. During the subsequent 2 days, the urinary output pped to 400 ml./24 hours, and she continued to have nausea and vomiting an elevation of blood urea nitrogen. However, the urine output subsetly returned to normal, and the patient made an uneventful recovery and discharged home on the 25th hospital day.

Discussion

ABLE 6 summarizes the results of our studies with the use of THAM in 5 ents. As may be seen, these patients manifested three different types of l disease: namely, postpartum bilateral renal cortical necrosis, acute and nic glomerulonephritis, and diabetic intracapillary glomerulonephrosclе-. All of these subjects had severe impairment of renal functions as manifested clinically by oliguria, elevated blood urea nitrogen, and abnormal urina findings. Two of these patients were anuric (less than 400 ml. of urine per hours), and two others were oliguric (less than 1000 ml. of urine per 24 hou Thirty-six gm. of THAM dissolved in 5 per cent dextrose and water was i

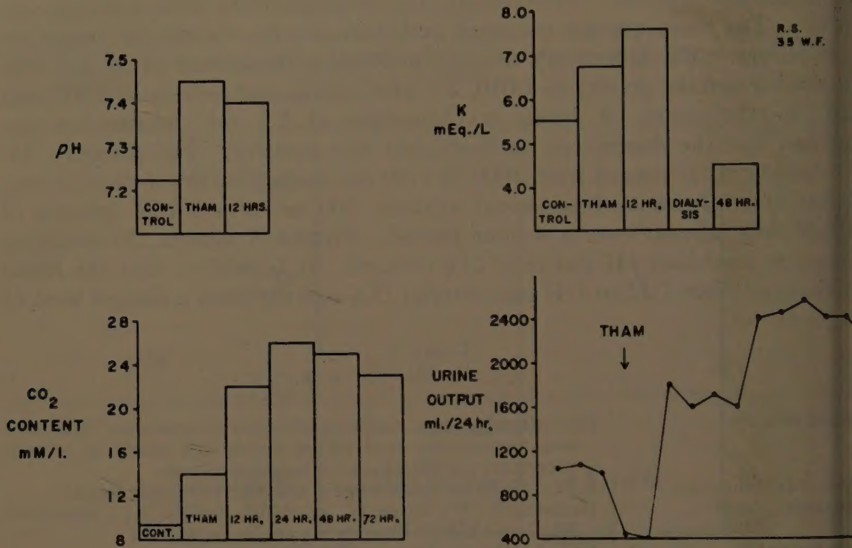


FIGURE 5. Alterations in the blood *p*H and bicarbonate level, serum concentratio potassium, and the urine flow after the administration of THAM.

TABLE 6

SUMMARY OF FIVE PATIENTS WITH SEVERE RENAL ACIDOSIS TREATED WITH THAM

Patient	Diagnosis	Urine output (ml./24 hr.)	Amount THAM (Gm.)	Duration of infusion	Δ *p*H	Change bicar-bonate (mM/l.)	Complication
G.S.	Intracapillary glomerulo-nephrosclerosis	100	36	12 hours	0.44	22.0	None
R.S.	Bilateral cortical necrosis	1100	36	4 hours	0.20	7.0	Hyperkalemi and oliguri
W.J.	Acute glomerulonephritis	1600	36	24 hours	0.10	7.2	None
O.Z.	Chronic glomerulonephritis	800	36	4 hours	0.26	10.5	Hyperkalemi
W.Z.	Acute glomerulonephritis	150	9	30 min.	0.02	2.5	? Hyperkaler

fused in 4 of these subjects over a period of time ranging between 30 min. 24 hours. In 1 patient only 9 gm. of THAM was administered over a 30-r period. In all of these cases there was a substantial rise in the blood *p*H ar significant increase in the total CO_2 content of the blood. However, non e patients showed any clinical improvement. The increase in blood *p*H total CO_2 content appeared to persist for at least 48 hours in most of these ents and continued to remain elevated for a period of several days in 2 of subjects (W.J. and R.S.).

n three of these 5 subjects the administration of THAM resulted in 2 major plications. Hyperkalemia was noted within 12 to 24 hours following the ninistration of THAM. Despite the apparent relationship between the e of administration of THAM and the developement of hyperkalemia, one uld be cautious in attributing this complication to the infusion of THAM, 2 of these subjects were acutely ill and manifested a severe degree of renal ure that could have resulted per se in hyperkalemia and oliguria. However, patient (R.S), who had an adequate initial urine output and a normal se- potassium level, the administration of THAM resulted within 12 hours in uria and hyperkalemia and an elevation of blood urea nitrogen level. It is terest to note that this patient received 6 gm. of THAM as a 0.6 *M* solution er rapidly over a 4-hour period. The mechanism of hyperkalemia following rapid administration of THAM to patients with severe renal disease is not wn. In our studies with dogs we have noted that the administration of e amounts of THAM to dogs with bilateral ligation of the ureters resulted marked hyperkalemia.* It is possible that hyperkalemia results from a of potassium from the intracellular compartment to the extracellular e as a consequence of infusion of THAM. Since these patients have a ked impairment of renal function, they are unable to excrete the increased red load of potassium.

conclusion, the results of our studies indicate that THAM is an effective t in the prompt correction of metabolic acidosis. In comparison with um bicarbonate or lactate, it may also offer the possible advantage of being to buffer the intracellular hydrogen ion. The frequent occurrence of sodium tion and congestive heart failure in patients with renal disease obviously s the usefulness of alkalizing agents that contain sodium, such as sodium rbonate or sodium lactate. THAM may be therefore a more suitable agent e correction of acidosis in those subjects where sodium restriction is ad- ole. Extreme care should be exercised in the use of this agent because of possible development of hyperkalemia, oliguria, and other complications. suggest that a maximum dose of 500 mg./kg. (as 0.3 *M* solution in dextrose water) be infused slowly over a 24-hour period with careful measurements e urinary output, serum potassium, and blood urea nitrogen for 48 hours wing the administration of THAM.

Acknowledgment

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